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[illegible] fragment [illegible] the TSRN [illegible] antibody. The invention additionally provides methods in which the TSRN polypeptides, [illegible] and antibody are utilized in the detection and treatment of a broad range of pathological states [illegible] other uses.

NOVEL POLYNUCLEOTIDES ENCODING PROTEINS CONTAINING THROMBOSPONDIN TYPE 1 REPEATS

FIELD OF THE INVENTION

The invention relates to human polynucleotides and polypeptides containing thrombospondin repeat (TSR) domains, as well as vectors, host cells, antibodies and recombinant methods for producing the polypeptides and polynucleotides.

BACKGROUND OF THE INVENTION

Thrombospondins are multidomain proteins that contain several types of repeated sequence modules. One type of repeat module is termed thrombospondin type 1 repeat or TSR. Although this discrete structural motif was originally identified in the thrombosponsdin family of proteins, many proteins are now known to belong to the TSR superfamily. These proteins include, *e.g.*, the F-spondin, SCO-spondin, UNC-5, METH-1 and ADAMSTS proteins.

The TSR is approximately 60 amino acids in length. Each repeat is thought to be encoded by a separate exon, which suggests that each repeat makes an independently folded protein structure. TSRs can be subgrouped in multiple sequence alignments according to their length and location of cysteine residues.

Functional roles attributed to TSRs include cell attachment, spreading, motility, and proliferation, cytoskelatal organization, wound healing and angiogenesis. Moreover, many of the TSR superfamily proteins are expressed in the nervous systems during development and are thought to play roles in neuronal growth and patterning. In particular, the thrombospondin, METH-1 and ADAMTS families of proteins are potent inhibitors of angiogenesis. The ADAMTS proteins have also been implicated in cleavage of proteglycans and the control of organ shape during development. In addition, the thrombospondins have been implicated in the activation of both transforming growth factor- beta (TGF-β) precursors and TGF-β in a

SUMMARY OF THE INVENTION

The present invention is based in part upon the discovery of a novel human nucleic acid sequence encoding a polypeptide having thrombospondin-1 repeat (TSR) domains. The nucleic acids, polynucleotides, proteins and polypeptides, or fragments thereof described herein are collectively referred to as TSRX nucleic acids and polypeptides

Accordingly, in one aspect, the invention provides an isolated nucleic acid molecule that includes the sequence of SEQ ID NO:1 or SEQ ID NO:3, or a fragment, homolog, analog or derivative thereof. The nucleic acid can include, *e.g.*, a nucleic acid sequence encoding a polypeptide at least 85% identical to a polypeptide that includes the amino acid sequences of SEQ ID NO:2 or SEQ ID NO: 4. The nucleic acid can be, *e.g.*, a genomic DNA fragment, or a cDNA molecule.

Also included in the invention is a vector containing one or more of the nucleic acids described herein, and a cell containing the vectors or nucleic acids described herein.

The invention is also directed to host cells transformed with a vector comprising any of the nucleic acid molecules described above.

In another aspect, the invention includes a pharmaceutical composition that includes an TSRX nucleic acid and a pharmaceutically acceptable carrier or diluent.

In a further aspect, the invention includes a substantially purified TSRX polypeptide, *e.g.*, any of the TSRX polypeptides encoded by an TSRX nucleic acid, and fragments, homologs, analogs, and derivatives thereof. The invention also includes a pharmaceutical composition that includes an TSRX polypeptide and a pharmaceutically acceptable carrier or diluent

In still a further aspect, the invention provides an antibody that binds specifically to an TSRX polypeptide. The antibody can be, *e.g.*, a monoclonal or polyclonal antibody, and fragments, homologs, analogs, and derivatives thereof. The invention also includes a pharmaceutical composition including TSRX antibody and a pharmaceutically acceptable carrier or diluent. The invention is also directed to isolated antibodies that bind to an epitope on a polypeptide encoded by any of the nucleic acid molecules described above

The invention further provides a method for producing a TSRX polypeptide by providing a cell containing an TSRX nucleic acid, e.g., a vector that includes a TSRX nucleic acid, and culturing the cell under conditions sufficient to express the TSRX polypeptide encoded by the nucleic acid. The expressed TSRX polypeptide is then recovered from the cell. Preferably, the cell produces little or no endogenous TSRX polypeptide. The cell can be, e.g., a prokaryotic cell or eukaryotic cell.

The invention is also directed to methods of identifying a TSRX polypeptide or nucleic acid in a sample by contacting the sample with a compound that specifically binds to the polypeptide or nucleic acid, and detecting complex formation, if present.

The invention further provides methods of identifying a compound that modulates the activity of a TSRX polypeptide by contacting a TSRX polypeptide with a compound and determining whether the TSRX polypeptide activity is modified.

The invention is also directed to compounds that modulate TSRX polypeptide activity identified by contacting a TSRX polypeptide with the compound and determining whether the compound modifies activity of the TSRX polypeptide, binds to the TSRX polypeptide, or binds to a nucleic acid molecule encoding a TSRX polypeptide.

In an another aspect, the invention provides a method of determining the presence of or predisposition of a TSRX-associated disorder in a subject. The method includes providing a sample from the subject and measuring the amount of TSRX polypeptide in the subject sample. The amount of TSRX polypeptide in the subject sample is then compared to the amount of TSRX polypeptide in a control sample. An alteration in the amount of TSRX polypeptide in the subject protein sample relative to the amount of TSRX polypeptide in the control protein sample indicates the subject has a tissue proliferation-associated condition. A control sample is preferably taken from a matched individual, i.e., an individual of similar age, sex, or other general condition but who is not suspected of having a tissue proliferation-associated condition. Alternatively, the control sample may be taken from the subject at a time when the subject is not suspected of having a tissue proliferation-associated disorder. In some embodiments, the TSRX is detected using a TSRX antibody.

of the TSRX nucleic acid in the subject nucleic acid sample. The amount of TSRX nucleic acid sample in the subject nucleic acid is then compared to the amount of a TSRX nucleic acid in a control sample. An alteration in the amount of TSRX nucleic acid in the sample relative to the amount of TSRX in the control sample indicates the subject has a tissue proliferation-associated disorder.

In a still further aspect, the invention provides a method of treating or preventing or delaying a TSRX-associated disorder. The method includes administering to a subject in which such treatment or prevention or delay is desired a TSRX nucleic acid, a TSRX polypeptide, or an TSRX antibody in an amount sufficient to treat, prevent, or delay a tissue proliferation-associated disorder in the subject.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In the case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

Other features and advantages of the invention will be apparent from the following detailed description and claims.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a representation of SDS-PAGE analysis showing expression of TSRX74 in [illegible] cells.

DETAILED DESCRIPTION OF THE INVENTION

The invention provides novel nucleotides and polypeptides containing [illegible] TSRX domains isolated from human mammary tissue.

polynucleotides" and the corresponding encoded polypeptide is referred to as a "TSRX polypeptide" or "TSRX protein". Unless indicated otherwise, "TSRX" is meant to refer to any of the novel sequences disclosed herein.

TSRX nucleic acids, and their encoded polypeptides, according to the invention are useful in a variety of applications and contexts. For example, TSRX nucleic acids and polypeptides can be used to identify proteins that are members of the TSR superfamily. The TSRX nucleic acids and polypeptides can also be used to screen for molecules which inhibit or enhance TSRX activity or function. Specifically, the nucleic acids and polypeptides according to the invention may be used as targets for the identification of small molecules that modulate or inhibit, e.g., angiogenesis or neuronal development. These molecules can be used to treat, e.g., cancer, rheumatoid arthritis and ocular neovascularisation in mammals, e.g. humans.

In addition, various TSRX nucleic acids and polypeptides according to the invention are useful, inter alia, as novel members of the protein families according to the presence of domains and sequence relatedness to previously described proteins. For example, the TSRX nucleic acids and their encoded polypeptides include structural motifs that are characteristic of proteins belonging to the TSR superfamily. Proteins belonging to this superfamily of proteins have been implicated in modulating and inhibiting angiogenesis. Angiogenesis is important for the growth of solid tumors. Thus, the TSRX nucleic acids and polypeptides, antibodies and related compounds according to the invention will be useful in therapeutic applications implicated in various cancers. In addition, TSRX nucleic acids, polypeptides, antibodies and related compounds of the invention may be used to modulate neuronal development, control organ shape during development, and facilitate the cleavage of proteoglycans.

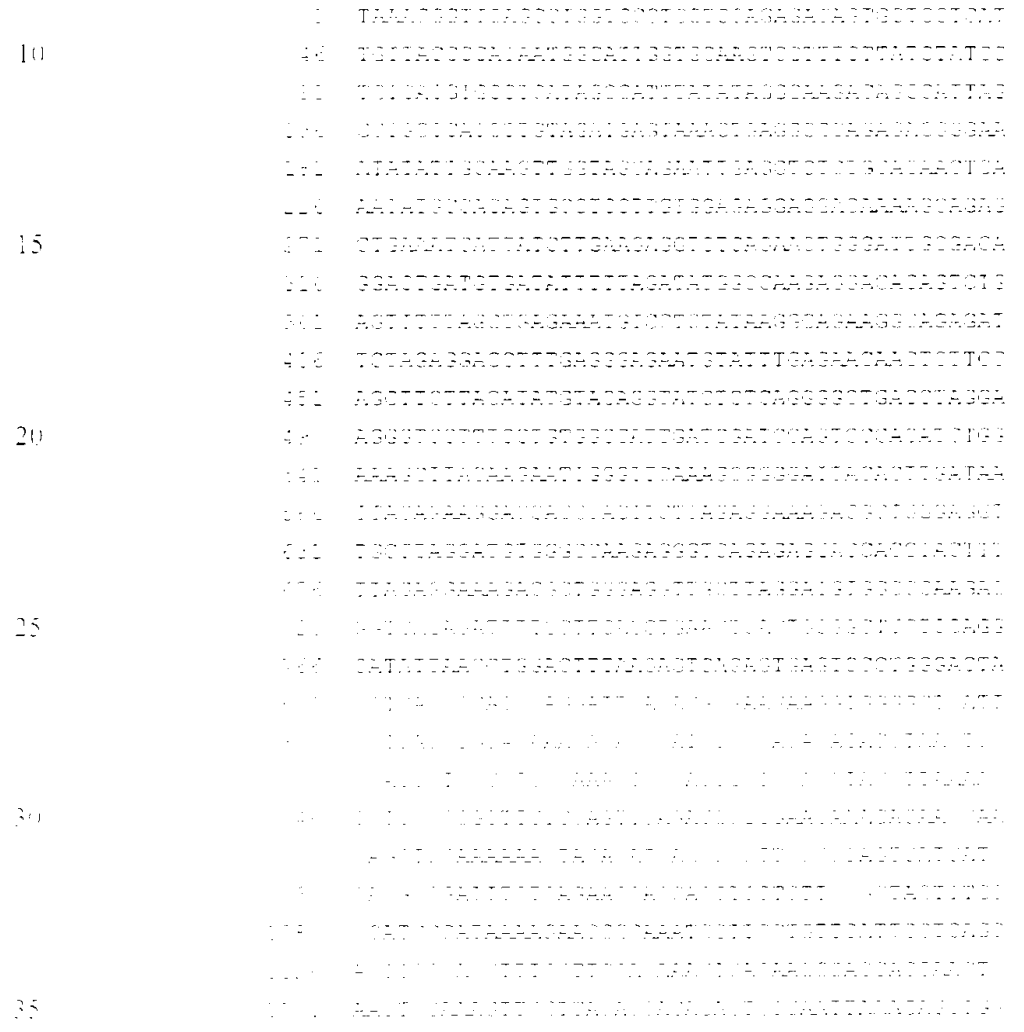
Tissue expression analysis as described in EXAMPLE 6 below demonstrates the relative absence of TSRX nucleic acids in various cancers, suggesting a potential therapeutic applications of TSRX nucleic acids and polypeptides either as a negative diagnostic marker for cancer or in the treatment of cancer. However, tissue expression analysis in surgical specimen indicate TSRX nucleic acids are more highly expressed in breast cancer metastases as compared to primary breast cancer. Accordingly, TSRX antibodies, antisense or other

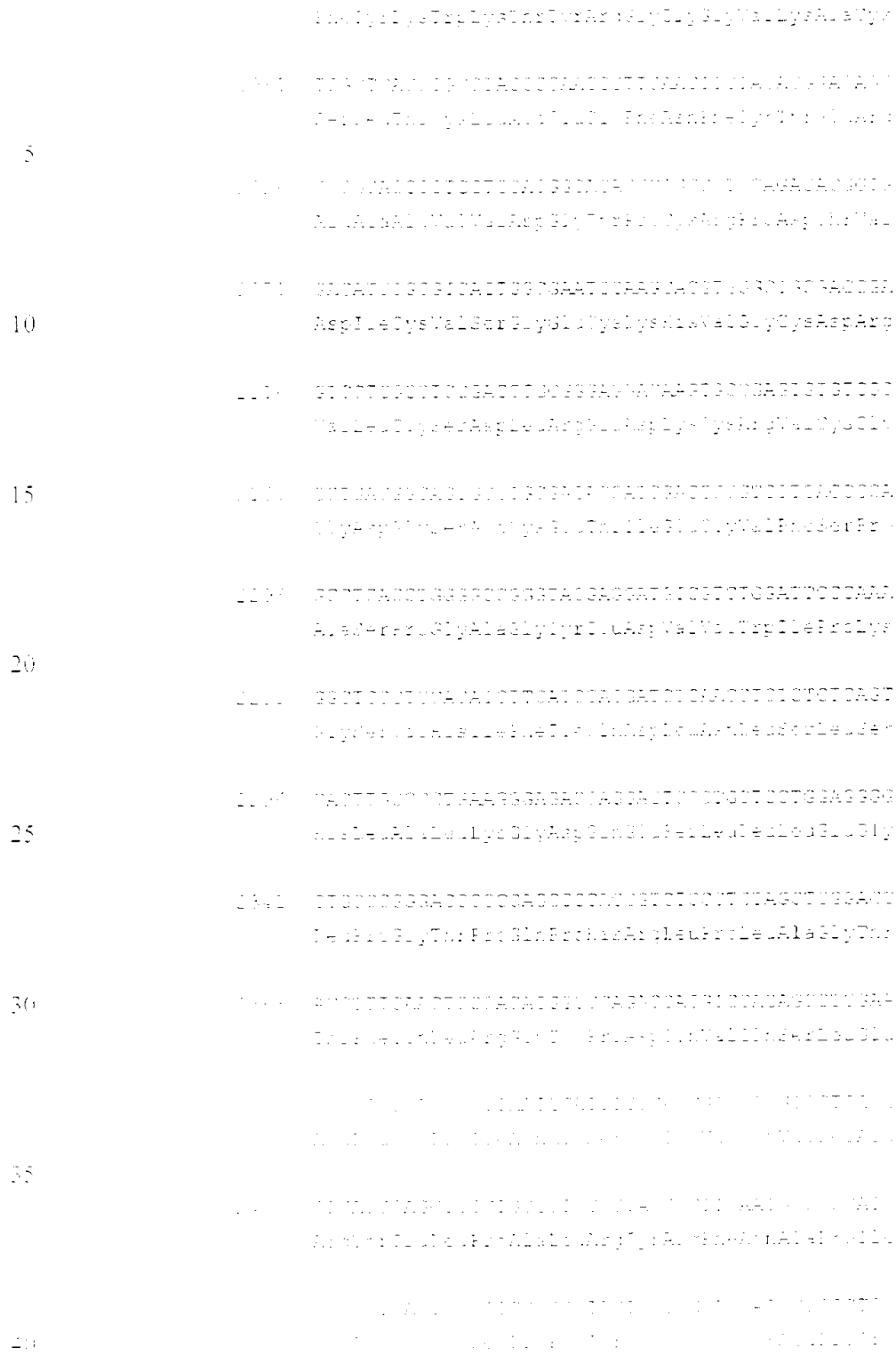
Additional utilities for TSRX nucleic acids and polypeptides according to the invention are disclosed herein.

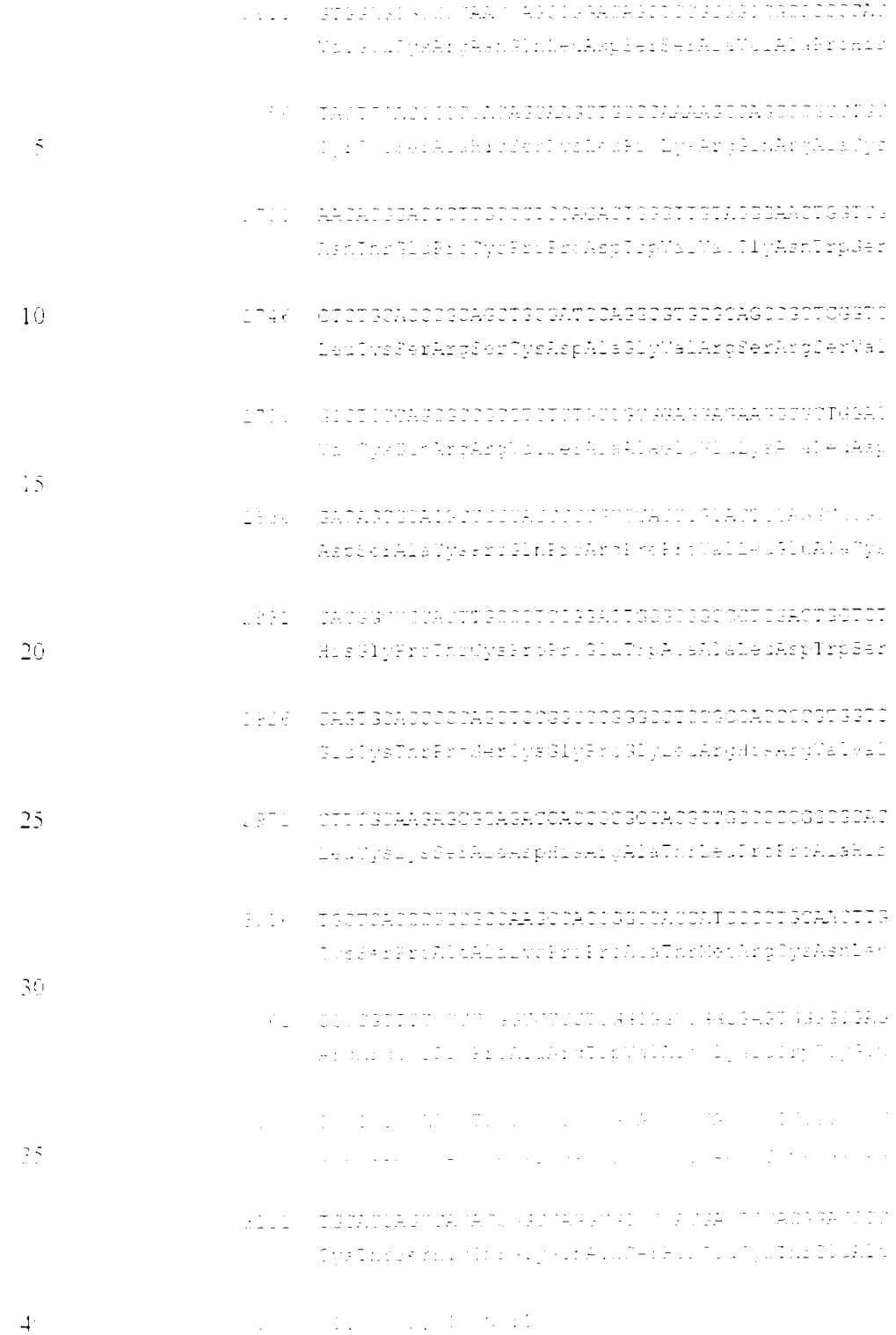
TSR1

A TSR1 nucleic acid and polypeptide according to the invention includes the nucleic acid and encoded polypeptide sequence of a sequence named 17897469.0.7. The predicted open reading frame codes for a 634 amino acid long secreted protein.

The 17897469.0.7 nucleic acid and encoded polypeptide has the following sequence:







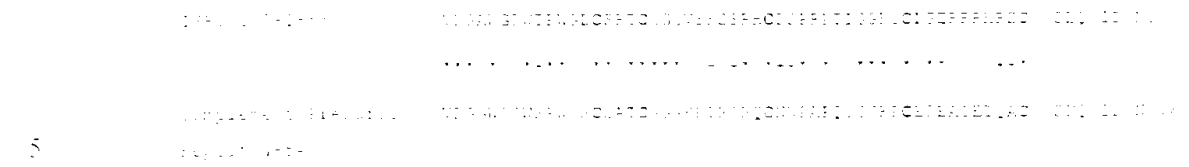
The disclosed TSR1 polypeptide sequence is expressed in mammary tissue and is predicted by the PSORT program to localize extracellularly. A putative signal sequence was predicted by SignalP, with cleavage occurring between amino acid residues 21 and 22. Searches in protein databases (e.g., BLASTP) showed similarity to human METH-2, a member of the TSR superfamily (Gen Bank Acc. No.:AAD48081). Specifically, the TSR1 polypeptide sequence has 127 of 341 residues (37%) identical to, and 185 of 341 residues (54%) positive with, the 890 residue human METH-2 protein. In addition, the TSR1 polypeptide also has an overall 61% identity to another member of the TSR superfamily, the Zn Metalloprotease ADAM-TS6 protein (GenBank Acc. No.: AAD5637).

PFAM HMM Domain analysis of TSR1 identified five regions within the polypeptide sequence as putative TSR domains. These regions correspond to TSR1 amino acid residues [illegible], 413-473, 477-533, 535-591 and 596-630. Table 1 is illustrative of a sequence alignment of TSR1 with the consensus TSR sequence from the PFAM HMM database. This region corresponds to amino acids 140-190 of TSR 1. Plus signs indicate similarity based on conservative amino acids substitutions and asterisks indicate identity. Consistent with other known members of the TSR superfamily of proteins, TSR1 contains a TSR module characterized by conserved cysteine residues as illustrated by bold in Table 1.

Table 1

[illegible]

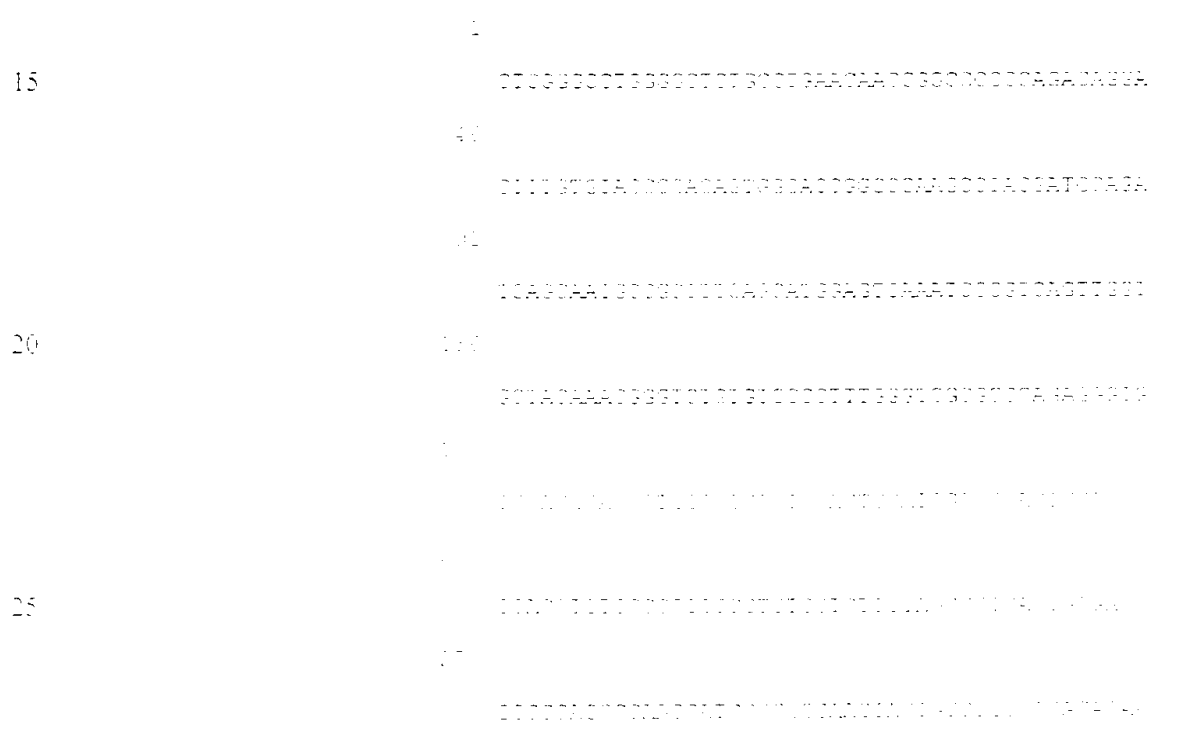
In addition to the PFAM HMM domain analysis, PRODOM analysis showed similarity to Complement Precursor Repeat, also a member of the TSR superfamily. Specifically, the TSR1 polypeptide sequence showed 49% identity to and 62% positive with Complement Precursor Repeat. The sequence alignment of amino acid residues 135-185 of a TSR1 polypeptide with amino acids residues 4-54 of Complement Precursor repeat is shown in Table 2. Plus signs indicate similarity based on conservative amino acids substitutions and asterisks

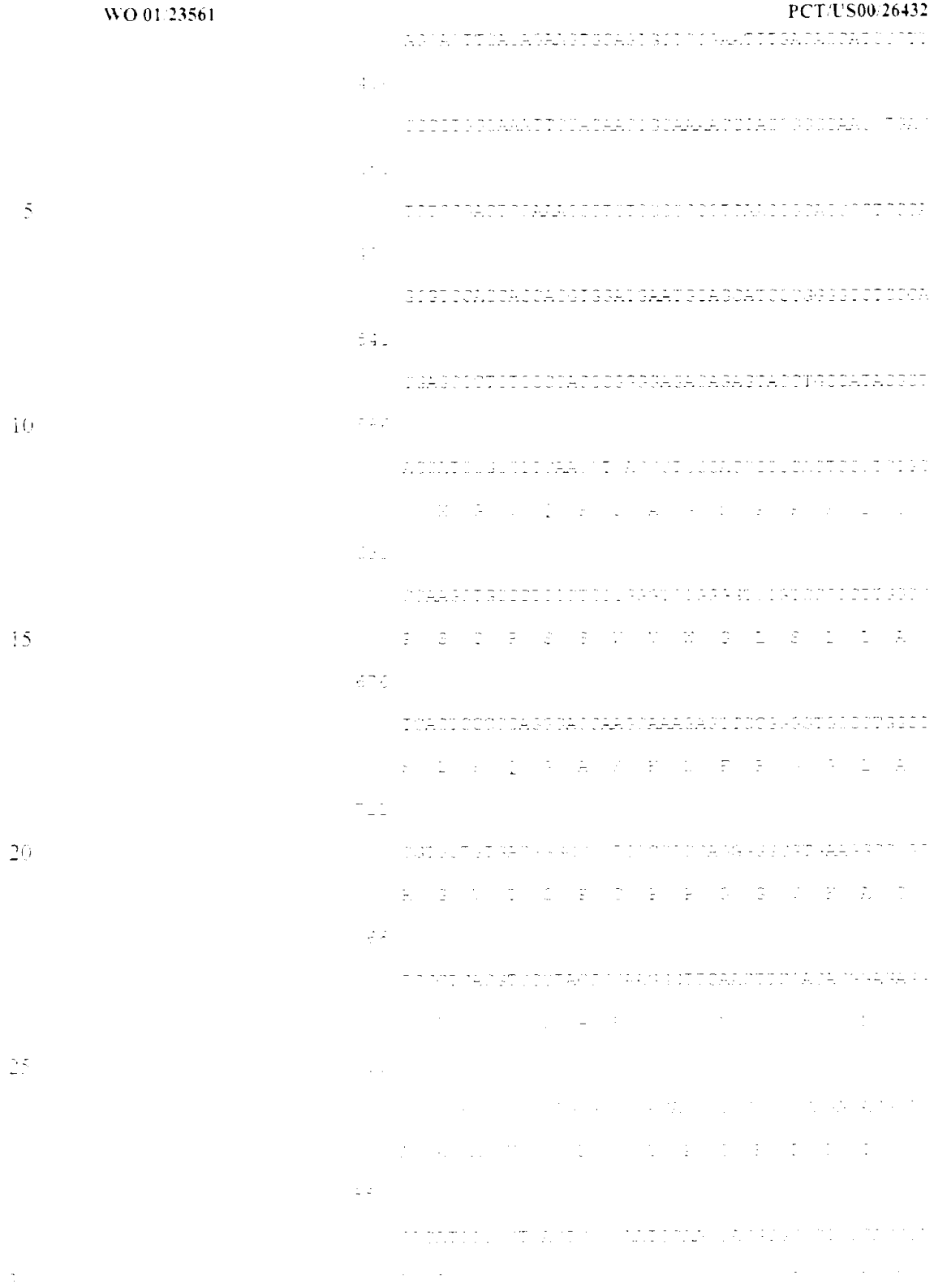


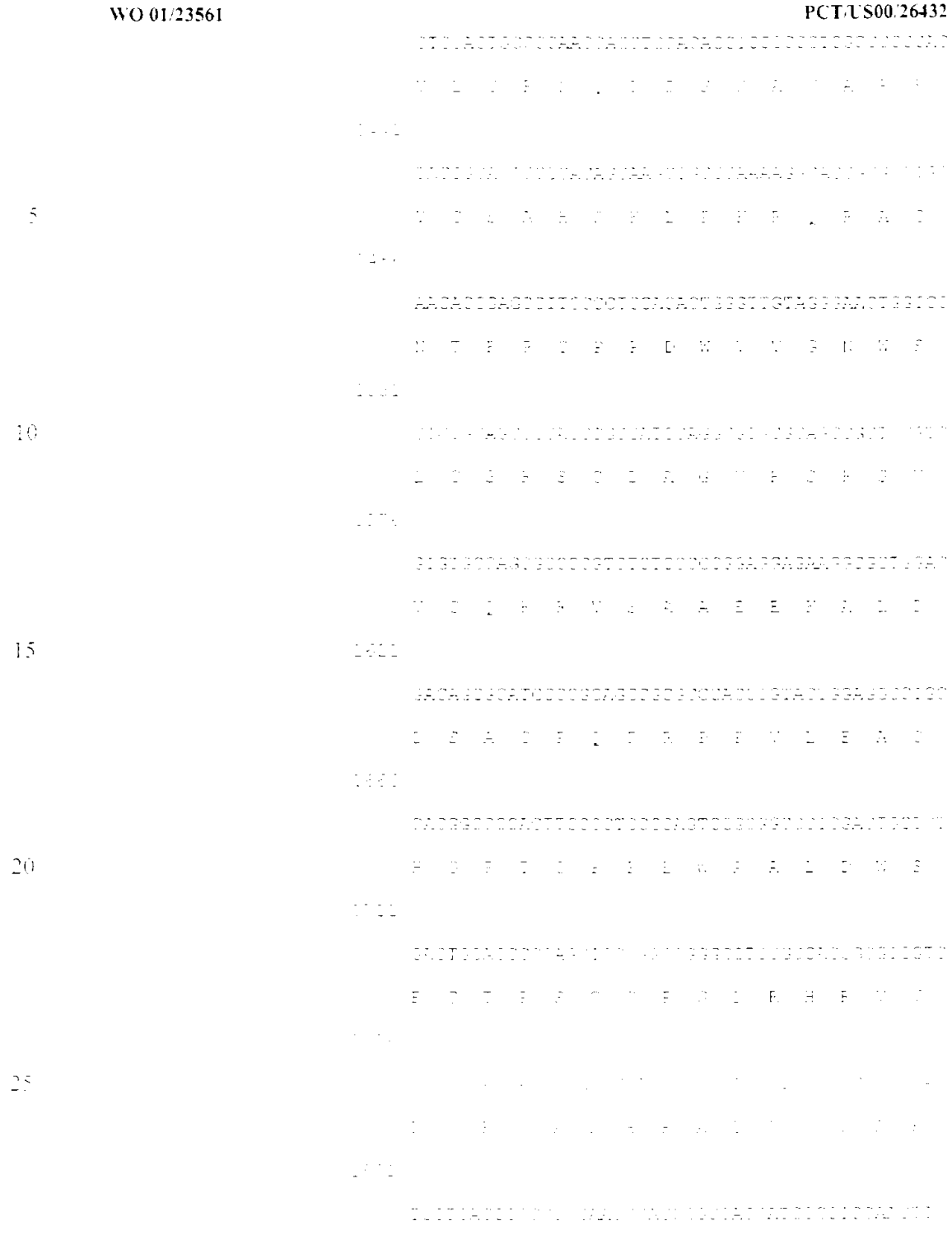
TSR2

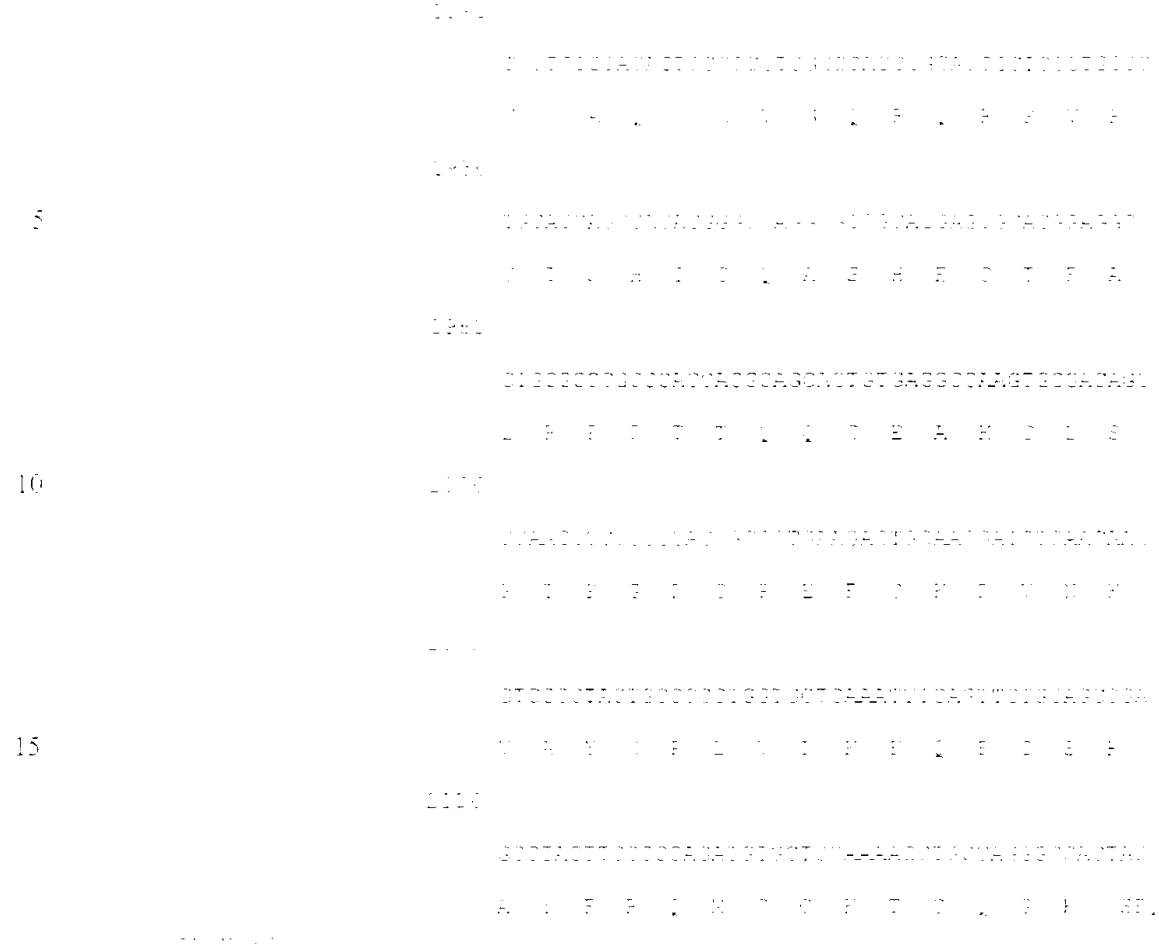
A TSR2 nucleic acid and polypeptide according to the invention includes the nucleic acid and encoded polypeptide sequence of a sequence named 17897469.0.187. The predicted open reading frame codes for a 523 amino acid long polypeptide. The calculated molecular weight of the protein is 56126.2 daltons.

The 17897469.0.187 nucleic acid and encoded polypeptide has the following sequence:







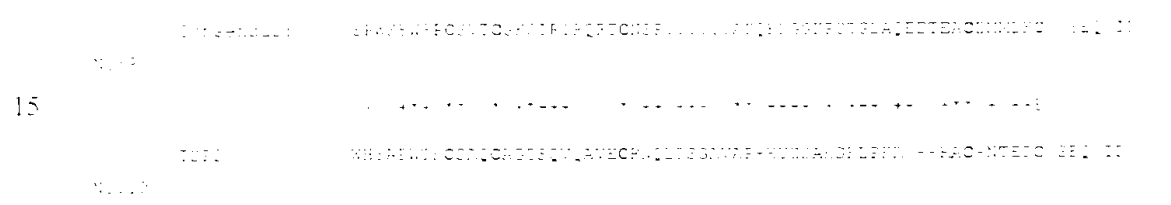


The disclosed TSR2 polypeptide sequence is expressed in mammary tissue, fetal brain and fetal lung and is predicted by the PSORT program to localize in the plasma membrane. A putative signal sequence was predicted by SignalP, with cleavage occurring between amino acid residues 36 and 37. Searches in sequence databases (e.g., BLASTP and BLASTX)

(GenBank Acc. No.: O95428). TSP2 nucleic acid and polypeptide has been identified as being a member of the human ADAMTS family of proteins.

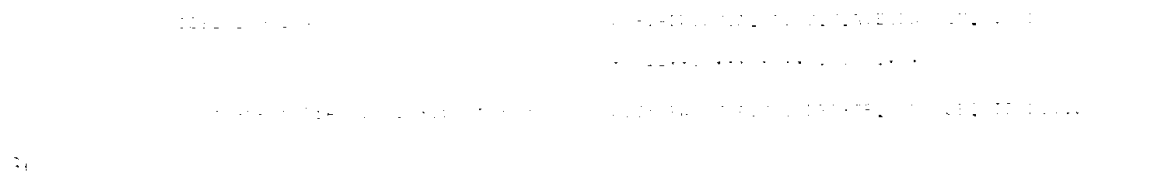
PFAM HMM Domain analysis of TSR2 identified four regions within the polypeptide sequence as putative TSR domains. These regions correspond to TSR1 amino acid residues 249-304, 308-364, 366-422 and 427-477. Table 1 below is illustrative of a sequence alignment of TSR2 with the consensus TSR sequence from the PFAM HMM database. This region corresponds to amino acids 249-304 of TSR 2. Plus signs indicate similarity based on conservative amino acids substitutions and asterisks indicate identity. Consistent with other known members of the TSR superfamily of proteins, TSR2 contains a TSR module characterized by conserved cysteine residues as illustrated by bold in Table 3.

Table 3



In addition to the PFAM HMM domain analysis, PRODOM analysis showed similarity to Complement Precursor Repeat, also a member of TSR superfamily. Specifically, the TSR2 polypeptide sequence showed 40% identity to and 60% positive with Complement Precursor Repeat. Sequence alignment of amino acid residues 249- 274 of a TSR2 polypeptide with amino acids 8-34 of Complement Precursor Repeat is shown in Table 2 below. Plus signs indicate similarity based on conservative amino acids substitutions and asterisks indicate identity. Conserved cysteine residues are illustrated by bold

Table 4



TSRX Nucleic Acids

The nucleic acids of the invention include those that encode a TSRX polypeptide or protein. As used herein, the terms polypeptide and protein are interchangeable.

In some embodiments, a TSRX nucleic acid encodes a mature TSRX polypeptide. As used herein, a "mature" form of a polypeptide or protein described herein relates to the product of a naturally occurring polypeptide or precursor form or proprotein. The naturally occurring polypeptide, precursor or proprotein includes, by way of nonlimiting example, the full length gene product, encoded by the corresponding gene. Alternatively, it may be defined as the polypeptide, precursor or proprotein encoded by an open reading frame described herein. The product "mature" form arises, again by way of nonlimiting example, as a result of one or more naturally occurring processing steps that may take place within the cell in which the gene product arises. Examples of such processing steps leading to a "mature" form of a polypeptide or protein include the cleavage of the N-terminal methionine residue encoded by the initiation codon of an open reading frame, or the proteolytic cleavage of a signal peptide or leader sequence. Thus a mature form arising from a precursor polypeptide or protein that has residues 1 to N, where residue 1 is the N-terminal methionine, would have residues 2 through N remaining after removal of the N-terminal methionine. Alternatively, a mature form arising from a precursor polypeptide or protein having residues 1 to N, in which an N-terminal signal sequence from residue 1 to residue M is cleaved, would have the residues from residue M+1 to residue N remaining. Further as used herein, a "mature" form of a polypeptide or protein may arise from a step of post-translational modification other than a proteolytic cleavage event. Such additional processes include, by way of non-limiting example, glycosylation, myristoylation or phosphorylation. In general, a mature polypeptide or protein may result from the operation of only one of these processes, or a combination of any of them.

Among the TSRX nucleic acids is the nucleic acid whose sequence is provided in SEQ ID NO: 1 and 3, or a fragment thereof. Additionally, the invention includes mutant or variant nucleic acids of SEQ ID NO: 1 and 3, or a fragment thereof, any of whose bases may be changed from the corresponding base shown in SEQ ID NO: 1 and 3, while still encoding a protein that maintains at least one of its TSRX-like activities and physiological functions (i.e.,

derivatives, analogs and homologs thereof. The invention additionally includes nucleic acids or nucleic acid fragments, or complements thereto, whose structures include chemical modifications.

One aspect of the invention pertains to isolated nucleic acid molecules that encode TSRX proteins or biologically active portions thereof. Also included are nucleic acid fragments sufficient for use as hybridization probes to identify TSRX-encoding nucleic acids (e.g., TSRX mRNA) and fragments for use as polymerase chain reaction (PCR) primers for the amplification or mutation of TSRX nucleic acid molecules. As used herein, the term "nucleic acid molecule" is intended to include DNA molecules (e.g., cDNA or genomic DNA), RNA molecules (e.g., mRNA), analogs of the DNA or RNA generated using nucleotide analogs, and derivatives, fragments and homologs thereof. The nucleic acid molecule can be single-stranded or double-stranded, but preferably is double-stranded DNA.

"Probes" refer to nucleic acid sequences of variable length, preferably between at least about 10 nucleotides (nt), 100 nt, or as many as about, e.g., 6,000 nt, depending on use. Probes are used in the detection of identical, similar, or complementary nucleic acid sequences. Longer length probes are usually obtained from a natural or recombinant source, are highly specific and much slower to hybridize than oligomers. Probes may be single- or double-stranded and designed to have specificity in PCR, membrane-based hybridization technologies, or ELISA-like technologies.

An "isolated" nucleic acid molecule is one that is separated from other nucleic acid molecules that are present in the natural source of the nucleic acid. Examples of isolated nucleic acid molecules include, but are not limited to, recombinant DNA molecules contained in a vector, recombinant DNA molecules maintained in a heterologous host cell, partially or substantially purified nucleic acid molecules, and synthetic DNA or RNA molecules. Preferably, an "isolated" nucleic acid is free of sequences which naturally flank the nucleic acid (i.e., sequences located at the 5' and 3' ends of the nucleic acid) in the genomic DNA of the organism from which the nucleic acid is derived. For example, in various embodiments, the isolated TSRX nucleic acid molecule can contain less than about 50 kb, 25 kb, 5 kb, 4 kb, 3 kb, 2 kb, 1 kb, 0.5 kb or 0.1 kb of nucleotide sequences which naturally flank the nucleic acid molecule in genomic DNA of the cell from which the nucleic acid is derived. Moreover,

other cellular material or culture medium when produced by recombinant techniques, or of chemical precursors or other chemicals when chemically synthesized.

A nucleic acid molecule of the present invention, e.g., a nucleic acid molecule having the nucleotide sequence of SEQ ID NO: 1 and 3, or a complement of any of this nucleotide sequence, can be isolated using standard molecular biology techniques and the sequence information provided herein. Using all or a portion of the nucleic acid sequence of SEQ ID NO: 1 and 3 as a hybridization probe, TSRX nucleic acid sequences can be isolated using standard hybridization and cloning techniques (e.g., as described in Sambrook *et al.*, eds., MOLECULAR CLONING: A LABORATORY MANUAL 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989; and Ausubel, *et al.*, eds. CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, New York, NY, 1993.)

A nucleic acid of the invention can be amplified using cDNA, mRNA or alternatively, genomic DNA, as a template and appropriate oligonucleotide primers according to standard PCR amplification techniques. The nucleic acid so amplified can be cloned into an appropriate vector and characterized by DNA sequence analysis. Furthermore, oligonucleotides corresponding to TSRX nucleotide sequences can be prepared by standard synthetic techniques, e.g., using an automated DNA synthesizer.

As used herein, the term "oligonucleotide" refers to a series of linked nucleotide residues, which oligonucleotide has a sufficient number of nucleotide bases to be used in a PCR reaction. A short oligonucleotide sequence may be based on, or designed from, a genomic or cDNA sequence and is used to amplify, confirm, or reveal the presence of an identical, similar or complementary DNA or RNA in a particular cell or tissue. Oligonucleotides comprise portions of a nucleic acid sequence having about 10 nt, 50 nt, or 100 nt in length, preferably about 15 nt to 30 nt in length. In one embodiment, an oligonucleotide comprising a nucleic acid molecule less than 100 nt in length would further comprise at least 6 contiguous nucleotides of SEQ ID NO: 1 and 3, or a complement thereof. Oligonucleotides may be chemically synthesized and may be used as probes.

In another embodiment, an isolated nucleic acid molecule of the invention includes a nucleic acid molecule that is a complement of the nucleotide sequence shown in SEQ ID NO: [illegible]. In one embodiment, an isolated nucleic acid molecule of the invention comprises

complementary to the nucleotide sequence shown in SEQ ID NO: 1 and 3 is one that is sufficiently complementary to the nucleotide sequence shown in SEQ ID NO: 1 and 3 that it can hydrogen bond with little or no mismatches to the nucleotide sequence shown in SEQ ID NO: 1 and 3, thereby forming a stable duplex.

As used herein, the term "complementary" refers to Watson-Crick or Hoogsteen base pairing between nucleotide units of a nucleic acid molecule, and the term "binding" means the physical or chemical interaction between two polypeptides or compounds or associated polypeptides or compounds or combinations thereof. Binding includes ionic, non-ionic, Von der Waals, hydrophobic interactions, etc. A physical interaction can be either direct or indirect. Indirect interactions may be through or due to the effects of another polypeptide or compound. Direct binding refers to interactions that do not take place through, or due to, the effect of another polypeptide or compound, but instead are without other substantial chemical intermediates.

Moreover, the nucleic acid molecule of the invention can comprise only a portion of the nucleic acid sequence of SEQ ID NO: 1 and 3, e.g., a fragment that can be used as a probe or primer, or a fragment encoding a biologically active portion of TSRX. Fragments provided herein are defined as sequences of at least 6 (contiguous) nucleic acids or at least 4 (contiguous) amino acids, a length sufficient to allow for specific hybridization in the case of nucleic acids or for specific recognition of an epitope in the case of amino acids, respectively, and are at most some portion less than a full length sequence. Fragments may be derived from any contiguous portion of a nucleic acid or amino acid sequence of choice. Derivatives are nucleic acid sequences or amino acid sequences formed from the native compounds either directly or by modification or partial substitution. Analogs are nucleic acid sequences or amino acid sequences that have a structure similar to, but not identical to, the native compound but differs from it in respect to certain components or side chains. Analogs may be synthetic or from a different evolutionary origin and may have a similar or opposite metabolic activity compared to wild type.

Derivatives and analogs may be full length or other than full length, if the derivative or analog contains a modified nucleic acid or amino acid, as described below. Derivatives or analogs of the nucleic acids or proteins of the invention include, but are not limited to,

95%, 98%, or even 99% identity (with a preferred identity of 80-99%) over a nucleic acid or amino acid sequence of identical size or when compared to an aligned sequence in which the alignment is done by a computer homology program known in the art, or whose encoding nucleic acid is capable of hybridizing to the complement of a sequence encoding the aforementioned proteins under stringent, moderately stringent, or low stringent conditions. See e.g. Ausubel, *et al.*, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, New York, NY, 1993, and below. An exemplary program is the Gap program (Wisconsin Sequence Analysis Package, Version 8 for UNIX, Genetics Computer Group University Research Park, Madison, WI) using the default settings, which uses the algorithm of Smith and Waterman (Adv. Appl. Math., 1981, 2: 482-489, which is incorporated herein by reference in its entirety).

A "homologous nucleic acid sequence" or "homologous amino acid sequence," or variations thereof, refer to sequences characterized by a homology at the nucleotide level or amino acid level as discussed above. Homologous nucleotide sequences encode those sequences coding for isoforms of a TSRX polypeptide. Isoforms can be expressed in different tissues of the same organism as a result of, for example, alternative splicing of RNA. Alternatively, isoforms can be encoded by different genes. In the present invention, homologous nucleotide sequences include nucleotide sequences encoding for a TSRX polypeptide of species other than humans, including, but not limited to, mammals, and thus can include, e.g., mouse, rat, rabbit, dog, cat cow, horse, and other organisms. Homologous nucleotide sequences also include, but are not limited to, naturally occurring allelic variations and mutations of the nucleotide sequences set forth herein. A homologous nucleotide sequence does not, however, include the nucleotide sequence encoding human TSRX protein. Homologous nucleic acid sequences include those nucleic acid sequences that encode conservative amino acid substitutions (see below) in SEQ ID NO:2*n* (where *n* = 1 to 7), as well as a polypeptide having TSRX activity. Biological activities of the TSRX proteins are described below. A homologous amino acid sequence does not encode the amino acid sequence of a human TSRX polypeptide.

The nucleotide sequence determined from the cloning of the human TSRX gene allows for the generation of probes and primers designed for use in identifying and/or cloning TSRX

oligonucleotide. The oligonucleotide typically comprises a region of nucleotide sequence that hybridizes under stringent conditions to at least about 12, 25, 50, 100, 150, 200, 250, 300, 350 or 400 or more consecutive sense strand nucleotide sequence of SEQ ID NO: 1 and 3; or an anti-sense strand nucleotide sequence of SEQ ID NO: 1 and 3; or of a naturally occurring mutant of SEQ ID NO: 1 and 3.

Probes based on the human TSRX nucleotide sequence can be used to detect transcripts or genomic sequences encoding the same or homologous proteins. In various embodiments, the probe further comprises a label group attached thereto, *e.g.*, the label group can be a radioisotope, a fluorescent compound, an enzyme, or an enzyme co-factor. Such probes can be used as a part of a diagnostic test kit for identifying cells or tissue which misexpress a TSRX protein, such as by measuring a level of a TSRX-encoding nucleic acid in a sample of cells from a subject e.g., detecting TSRX mRNA levels or determining whether a genomic TSRX gene has been mutated or deleted.

A "polypeptide having a biologically active portion of TSRX" refers to polypeptides exhibiting activity similar, but not necessarily identical to, an activity of a polypeptide of the present invention, including mature forms, as measured in a particular biological assay, with or without dose dependency. A nucleic acid fragment encoding a "biologically active portion of TSRX" can be prepared by isolating a portion of SEQ ID NO: 1 and 3 that encodes a polypeptide having a TSRX biological activity (biological activities of the TSRX proteins are described below), expressing the encoded portion of TSRX protein (e.g., by recombinant expression *in vitro*) and assessing the activity of the encoded portion of TSRX. For example, a nucleic acid fragment encoding a biologically active portion of TSRX can optionally include an ATP-binding domain. In another embodiment, a nucleic acid fragment encoding a biologically active portion of TSRX includes one or more regions.

TSRX Variants

The invention further encompasses nucleic acid molecules that differ from the nucleotide sequences shown in SEQ ID NO: 1 and 3 due to the degeneracy of the genetic code. These nucleic acids thus encode the same TSRX protein as that encoded by the nucleotide sequence shown in SEQ ID NO: 1 and 3 (*i.e.*, the polypeptide of SEQ ID NO: 2 and 4). In

In addition to the human TSRX nucleotide sequence shown in SEQ ID NO: 1 and 3, it will be appreciated by those skilled in the art that DNA sequence polymorphisms that lead to changes in the amino acid sequences of TSRX may exist within a population (e.g., the human population). Such genetic polymorphism in the TSRX gene may exist among individuals within a population due to natural allelic variation. As used herein, the terms "gene" and "recombinant gene" refer to nucleic acid molecules comprising an open reading frame encoding a TSRX protein, preferably a mammalia TSRX protein. Such natural allelic variations can typically result in 1-5% variance in the nucleotide sequence of the TSRX gene. Any and all such nucleotide variations and resulting amino acid polymorphisms in TSRX that are the result of natural allelic variation and that do not alter the functional activity of TSRX are intended to be within the scope of the invention.

Moreover, nucleic acid molecules encoding TSRX proteins from other species, and thus that have a nucleotide sequence that differs from the human sequence of SEQ ID NO: 1 and 3are intended to be within the scope of the invention. Nucleic acid molecules corresponding to natural allelic variants and homologues of the TSRX cDNAs of the invention can be isolated based on their homology to the huma TSRX nucleic acids disclosed herein using the human cDNAs, or a portion thereof, as a hybridization probe according to standard hybridization techniques under stringent hybridization conditions. For example, a soluble huma TSRX cDNA can be isolated based on its homology to human membrane-bound TSRX. Likewise, a membrane-bound huma TSRX cDNA can be isolated based on its homology to soluble huma TSRX.

Accordingly, in another embodiment, an isolated nucleic acid molecule of the invention is at least 6 nucleotides in length and hybr dizes under stringent conditions to the nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO: 1 and 3. In another embodiment, the nucleic acid is at least 10, 25, 50, 100, 250, 500 or 750 nucleotides in length. In another embodiment, an isolated nucleic acid molecule of the invention hybridizes to the coding region. As used herein, the term "hybridizes under stringent conditions" is intended to describe conditions for hybridization and washing under which nucleotide sequences at least 60% homologous to each other typically remain hybridized to each other.

Homologs (i.e., nucleic acids encoding TSRX proteins derived from species other than

stringency hybridization with all or a portion of the particular human sequence as a probe using methods well known in the art for nucleic acid hybridization and cloning.

As used herein, the phrase "stringent hybridization conditions" refers to conditions under which a probe, primer or oligonucleotide will hybridize to its target sequence, but to no other sequences. Stringent conditions are sequence-dependent and will be different in different circumstances. Longer sequences hybridize specifically at higher temperatures than shorter sequences. Generally, stringent conditions are selected to be about 5°C lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength and pH. The Tm is the temperature (under defined ionic strength, pH and nucleic acid concentration) at which 50% of the probes complementary to the target sequence hybridize to the target sequence at equilibrium. Since the target sequences are generally present at excess, at Tm, 50% of the probes are occupied at equilibrium. Typically, stringent conditions will be those in which the salt concentration is less than about 1.6 M sodium ion, typically about 0.01 to 1.0 M sodium ion (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30°C for short probes, primers or oligonucleotides (e.g., 10 nt to 50 nt) and at least about 60°C for longer probes, primers and oligonucleotides. Stringent conditions may also be achieved with the addition of destabilizing agents, such as formamide.

Stringent conditions are known to those skilled in the art and can be found in CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6. Preferably, the conditions are such that sequences at least about 65%, 70%, 75%, 85%, 90%, 95%, 98%, or 99% homologous to each other typically remain hybridized to each other. A non-limiting example of stringent hybridization conditions is hybridization in a high salt buffer comprising 6X SSC, 50 mM Tris-HCl (pH 7.5), 1 mM EDTA, 0.02% PVP, 0.02% Ficoll, 0.02% BSA, and 500 mg/ml denatured salmon sperm DNA at 65°C. This hybridization is followed by one or more washes in 0.2X SSC, 0.01% BSA at 50°C. An isolated nucleic acid molecule of the invention that hybridizes under stringent conditions to the sequence of SEQ ID NO: 1 and 3 corresponds to a naturally-occurring nucleic acid molecule. As used herein, a "naturally-occurring" nucleic acid molecule refers to an RNA or DNA molecule having a nucleotide sequence that occurs in nature (e.g., encodes a natural protein).

In a gene pool of a human, a nucleic acid sequence that is hybridizable to the nucleic

non-limiting example of moderate stringency hybridization conditions are hybridization in 6X SSC, 5X Denhardt's solution, 0.5% SDS and 100 mg ml denatured salmon sperm DNA at 55°C, followed by one or more washes in 1X SSC, 0.1% SDS at 37°C. Other conditions of moderate stringency that may be used are well known in the art. See, e.g., Ausubel *et al.* (eds.), 1993, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, NY, and Kriegler, 1990, GENE TRANSFER AND EXPRESSION: A LABORATORY MANUAL, Stockton Press, NY.

In a third embodiment, a nucleic acid that is hybridizable to the nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO: 1 and 3, or fragments, analogs or derivatives thereof, under conditions of low stringency, is provided. A non-limiting example of low stringency hybridization conditions are hybridization in 35% formamide, 5X SSC, 50 mM Tris-HCl (pH 7.5), 5 mM EDTA, 0.02% PVP, 0.02% Ficoll, 0.2% BSA, 100 mg ml denatured salmon sperm DNA, 10% (wt vol) dextran sulfate at 40°C, followed by one or more washes in 2X SSC, 25 mM Tris-HCl (pH 7.4), 5 mM EDTA, and 0.1% SDS at 50°C. Other conditions of low stringency that may be used are well known in the art (e.g., as employed for cross-species hybridizations). See, e.g., Ausubel *et al.* (eds.), 1993, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, NY, and Kriegler, 1990, GENE TRANSFER AND EXPRESSION, A LABORATORY MANUAL, Stockton Press, NY; Shilo and Weinberg, 1981, *Proc Natl Acad Sci USA* 78: 6789-6792

Conservative mutations

In addition to naturally-occurring allelic variants of the TSRX sequence that may exist in the population, the skilled artisan will further appreciate that changes can be introduced by mutation into the nucleotide sequence of SEQ ID NO: 1 and 3, thereby leading to changes in the amino acid sequence of the encoded TSRX protein, without altering the functional ability of the TSRX protein. For example, nucleotide substitutions leading to amino acid substitutions at "non-essential" amino acid residues can be made in the sequence of SEQ ID NO: 1 and 3. A "non-essential" amino acid residue is a residue that can be altered from the wild-type sequence of TSRX without altering the biological activity, whereas an "essential" amino acid residue is required for biological activity. For example, amino acid residues that [illegible] TSRX proteins of the present invention, are predicted to be

Another aspect of the invention pertains to nucleic acid molecules encoding TSRX proteins that contain changes in amino acid residues that are not essential for activity. Such TSRX proteins differ in amino acid sequence from SEQ ID NO: 2 and 4, yet retain biological activity. In one embodiment, the isolated nucleic acid molecule comprises a nucleotide sequence encoding a protein, wherein the protein comprises an amino acid sequence at least about 75% homologous to the amino acid sequence of SEQ ID NO: 2 and 4. Preferably, the protein encoded by the nucleic acid is at least about 80% homologous to SEQ ID NO: 2 and 4, more preferably at least about 90%, 95%, 98%, and most preferably at least about 99% homologous to SEQ ID NO: 2 and 4.

An isolated nucleic acid molecule encoding a TSRX protein homologous to the protein of SEQ ID NO: 2 and 4 can be created by introducing one or more nucleotide substitutions, additions or deletions into the nucleotide sequence of SEQ ID NO:2*n*-1 (wherein *n*= 1 to 7), such that one or more amino acid substitutions, additions or deletions are introduced into the encoded protein.

Mutations can be introduced into the nucleotide sequence of SEQ ID NO: 1 and 3 by standard techniques, such as site-directed mutagenesis and PCR-mediated mutagenesis. Preferably, conservative amino acid substitutions are made at one or more predicted non-essential amino acid residues. A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine). Thus, a predicted nonessential amino acid residue in TSRX is replaced with another amino acid residue from the same side chain family. Alternatively, in another embodiment, mutations can be introduced randomly along all or part of a TSRX coding sequence, such as by saturation mutagenesis, and the resultant mutants can be screened for TSRX biological activity to identify mutants that retain activity. Following

In one embodiment, a mutant TSRX protein can be assayed for (1) the ability to form protein-protein interactions with other TSRX proteins, other cell-surface proteins, or biologically active portions thereof, (2) complex formation between a mutant TSRX protein and a TSRX receptor; (3) the ability of a mutant TSRX protein to bind to an intracellular target protein or biologically active portion thereof; (e.g., avidin proteins); (4) the ability to bind TSRX protein; or (5) the ability to specifically bind an anti-TSRX protein antibody.

Antisense TSRX Nucleic Acids

Another aspect of the invention pertains to isolated antisense nucleic acid molecules that are hybridizable to or complementary to the nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO: 1 and 3, or fragments, analogs or derivatives thereof. An "antisense" nucleic acid comprises a nucleotide sequence that is complementary to a "sense" nucleic acid encoding a protein, e.g., complementary to the coding strand of a double-stranded cDNA molecule or complementary to an mRNA sequence. In specific aspects, antisense nucleic acid molecules are provided that comprise a sequence complementary to at least about 10, 25, 50, 100, 250 or 500 nucleotides or an entire TSRX coding strand, or to only a portion thereof. Nucleic acid molecules encoding fragments, homologs, derivatives and analogs of a TSRX protein of SEQ ID NO: 2 and 4, or antisense nucleic acids complementary to a TSRX nucleic acid sequence of SEQ ID NO: 1 and 3 are additionally provided.

In one embodiment, an antisense nucleic acid molecule is antisense to a "coding region" of the coding strand of a nucleotide sequence encoding TSRX. The term "coding region" refers to the region of the nucleotide sequence comprising codons which are translated into amino acid residues (e.g., the protein coding region of human TSRX corresponds to SEQ ID NO: 2 and 4). In another embodiment, the antisense nucleic acid molecule is antisense to a "noncoding region" of the coding strand of a nucleotide sequence encoding TSRX. The term "noncoding region" refers to 5' and 3' sequences which flank the coding region that are not translated into amino acids (i.e., also referred to as 5' and 3' untranslated regions).

Given the coding strand sequences encoding TSRX disclosed herein (e.g., SEQ ID NO: 1 and 3), antisense nucleic acids of the invention can be designed according to the rules of [illegible]. The antisense nucleic acid molecule can be

mRNA. For example, the antisense oligonucleotide can be complementary to the region surrounding the translation start site of TSRX mRNA. An antisense oligonucleotide can be, for example, about 5, 10, 15, 20, 25, 30, 35, 40, 45 or 50 nucleotides in length. An antisense nucleic acid of the invention can be constructed using chemical synthesis or enzymatic ligation reactions using procedures known in the art. For example, an antisense nucleic acid (e.g., an antisense oligonucleotide) can be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed between the antisense and sense nucleic acids, e.g., phosphorothioate derivatives and acridine substituted nucleotides can be used.

Examples of modified nucleotides that can be used to generate the antisense nucleic acid include: 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xanthine, 4-acetylcytosine, 5-(carboxyhydroxylmethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine. Alternatively, the antisense nucleic acid can be produced biologically using an expression vector into which a nucleic acid has been subcloned in an antisense orientation (i.e., RNA transcribed from the inserted nucleic acid will be of an antisense orientation to a target nucleic acid of interest, described further in the following subsection).

The antisense nucleic acid molecules of the invention are typically administered to a subject or generated *in situ* such that they hybridize with or bind to cellular mRNA and/or genomic DNA encoding a TSRX protein to thereby inhibit expression of the protein, e.g., by inhibiting transcription and/or translation. The hybridization can be by conventional

the major groove of the double helix. An example of a route of administration of antisense nucleic acid molecules of the invention includes direct injection at a tissue site. Alternatively, antisense nucleic acid molecules can be modified to target selected cells and then administered systemically. For example, for systemic administration, antisense molecules can be modified such that they specifically bind to receptors or antigens expressed on a selected cell surface, e.g., by linking the antisense nucleic acid molecules to peptides or antibodies that bind to cell surface receptors or antigens. The antisense nucleic acid molecules can also be delivered to cells using the vectors described herein. To achieve sufficient intracellular concentrations of antisense molecules, vector constructs in which the antisense nucleic acid molecule is placed under the control of a strong pol II or pol III promoter are preferred.

In yet another embodiment, the antisense nucleic acid molecule of the invention is an α-anomeric nucleic acid molecule. An α-anomeric nucleic acid molecule forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual β-units, the strands run parallel to each other (Gaultier *et al.* (1987) *Nucleic Acids Res* 15: 6625-6641). The antisense nucleic acid molecule can also comprise a 2'-o-methylribonucleotide (Inoue *et al.* (1987) *Nucleic Acids Res* 15: 6131-6148) or a chimeric RNA -DNA analogue (Inoue *et al.* (1987) *FEBS Lett* 215: 327-330).

Such modifications include, by way of nonlimiting example, modified bases, and nucleic acids whose sugar phosphate backbones are modified or derivatized. These modifications are carried out at least in part to enhance the chemical stability of the modified nucleic acid, such that they may be used, for example, as antisense binding nucleic acids in therapeutic applications in a subject.

TSRX Ribozymes and PNA moieties

In still another embodiment, an antisense nucleic acid of the invention is a ribozyme. Ribozymes are catalytic RNA molecules with ribonuclease activity that are capable of cleaving a single-stranded nucleic acid, such as a mRNA, to which they have a complementary region. Thus, ribozymes (e.g., hammerhead ribozymes (described in Haselhoff and Gerlach (1988) *Nature* 334:585-591)) can be used to catalytically cleave TSRX mRNA transcripts to thereby inhibit translation of TSRX mRNA. A ribozyme having specificity for a TSRX-encoding

RNA can be constructed in which the nucleotide sequence of the active site is complementary to the nucleotide sequence to be cleaved in a TSRX-encoding mRNA. See, e.g., Cech *et al.* U.S. Pat. No. 4,987,071; and Cech *et al.* U.S. Pat. No. 5,116,742. Alternatively, TSRX mRNA can be used to select a catalytic RNA having a specific ribonuclease activity from a pool of RNA molecules. See, e.g., Bartel *et al.* (1993) *Science* 261:1411-1418.

Alternatively, TSRX gene expression can be inhibited by targeting nucleotide sequences complementary to the regulatory region of the TSRX (e.g., the TSRX promoter and/or enhancers) to form triple helical structures that prevent transcription of the TSRX gene in target cells. See generally, Helene. (1991) *Anticancer Drug Des.* 6: 569-84; Helene. *et al.* (1992) *Ann. N.Y. Acad. Sci.* 660:27-36; and Maher (1992) *Bioassays* 14: 807-15.

In various embodiments, the nucleic acids of TSRX can be modified at the base moiety, sugar moiety or phosphate backbone to improve, e.g., the stability, hybridization, or solubility of the molecule. For example, the deoxyribose phosphate backbone of the nucleic acids can be modified to generate peptide nucleic acids (see Hyrup *et al.* (1996) *Bioorg Med Chem* 4: 5-23). As used herein, the terms "peptide nucleic acids" or "PNAs" refer to nucleic acid mimics, e.g., DNA mimics, in which the deoxyribose phosphate backbone is replaced by a pseudopeptide backbone and only the four natural nucleobases are retained. The neutral backbone of PNAs has been shown to allow for specific hybridization to DNA and RNA under conditions of low ionic strength. The synthesis of PNA oligomers can be performed using standard solid phase peptide synthesis protocols as described in Hyrup *et al.* (1996) above; Perry-O'Keefe *et al.* (1996) *PNAS* 93: 14670-675.

PNAs of TSRX can be used in therapeutic and diagnostic applications. For example, PNAs can be used as antisense or antigene agents for sequence-specific modulation of gene expression by, e.g., inducing transcription or translation arrest or inhibiting replication. PNAs of TSRX can also be used, e.g., in the analysis of single base pair mutations in a gene by, e.g., PNA directed PCR clamping; as artificial restriction enzymes when used in combination with other enzymes, e.g., S1 nucleases (Hyrup B. (1996) above); or as probes or primers for DNA sequence and hybridization (Hyrup *et al.* (1996), above; Perry-O'Keefe (1996), above).

In another embodiment, PNAs of TSRX can be modified, e.g., to enhance their stability or cellular uptake, by attaching lipophilic or other helper groups to PNA, by the

may combine the advantageous properties of PNA and DNA. Such chimeras allow DNA recognition enzymes, e.g., RNase H and DNA polymerases, to interact with the DNA portion while the PNA portion would provide high binding affinity and specificity. PNA-DNA chimeras can be linked using linkers of appropriate lengths selected in terms of base stacking, number of bonds between the nucleobases, and orientation (Hyrup (1996) above). The synthesis of PNA-DNA chimeras can be performed as described in Hyrup (1996) above and Finn *et al.* (1996) *Nucl Acids Res* 24: 3357-63. For example, a DNA chain can be synthesized on a solid support using standard phosphoramidite coupling chemistry, and modified nucleoside analogs, e.g., 5'-(4-methoxytrityl) amino-5'-deoxy-thymidine phosphoramidite, can be used between the PNA and the 5' end of DNA (Mag *et al.* (1989) *Nucl Acid Res* 17: 5973-88). PNA monomers are then coupled in a stepwise manner to produce a chimeric molecule with a 5' PNA segment and a 3' DNA segment (Finn *et al.* (1996) above). Alternatively, chimeric molecules can be synthesized with a 5' DNA segment and a 3' PNA segment. See, Petersen *et al.* (1975) *Bioorg Med Chem Lett* 5: 1119-11124.

In other embodiments, the oligonucleotide may include other appended groups such as peptides (e.g., for targeting host cell receptors *in vivo*), or agents facilitating transport across the cell membrane (see, *e.g.*, Letsinger *et al.*, 1989, *Proc. Natl. Acad. Sci. U.S.A.* 86:6553-6556; Lemaitre *et al.*, 1987, Proc. *Natl. Acad. Sci.* 84:648-652; PCT Publication No. W088/09810) or the blood-brain barrier (see, *e.g.*, PCT Publication No. W089/10134). In addition, oligonucleotides can be modified with hybridization triggered cleavage agents (See, *e.g.*, Krol *et al.*, 1988, *BioTechniques* 6:958-976) or intercalating agents. (See, *e.g.*, Zon, 1988, *Pharm. Res.* 5: 539-549). To this end, the oligonucleotide may be conjugated to another molecule, *e.g.*, a peptide, a hybridization triggered cross-linking agent, a transport agent, a hybridization-triggered cleavage agent, etc.

TSRX Polypeptides

A TSRX polypeptide of the invention includes the TSRX-like protein whose sequence is provided in SEQ ID NO: 2 and 4. The invention also includes a mutant or variant protein any of whose residues may be changed from the corresponding residue shown in SEQ ID NO: 2 and 4 while still encoding a protein that maintains its TSRX-like activities and physiological functions, or a functional fragment thereof. In some embodiments, up to 20% or more of the

In general, a TSRX -like variant that preserves TSRX-like function includes any variant in which residues at a particular position in the sequence have been substituted by other amino acids, and further include the possibility of inserting an additional residue or residues between two residues of the parent protein as well as the possibility of deleting one or more residues from the parent sequence. Any amino acid substitution, insertion, or deletion is encompassed by the invention. In favorable circumstances, the substitution is a conservative substitution as defined above.

One aspect of the invention pertains to isolated TSRX proteins, and biologically active portions thereof, or derivatives, fragments, analogs or homologs thereof. Also provided are polypeptide fragments suitable for use as immunogens to raise anti-TSRX antibodies. In one embodiment, native TSRX proteins can be isolated from cells or tissue sources by an appropriate purification scheme using standard protein purification techniques. In another embodiment, TSRX proteins are produced by recombinant DNA techniques. Alternative to recombinant expression, a TSRX protein or polypeptide can be synthesized chemically using standard peptide synthesis techniques.

An "isolated" or "purified" protein or biologically active portion thereof is substantially free of cellular material or other contaminating proteins from the cell or tissue source from which the TSRX protein is derived, or substantially free from chemical precursors or other chemicals when chemically synthesized. The language "substantially free of cellular material" includes preparations of TSRX protein in which the protein is separated from cellular components of the cells from which it is isolated or recombinantly produced. In one embodiment, the language "substantially free of cellular material" includes preparations of TSRX protein having less than about 30% (by dry weight) of non-TSRX protein (also referred to herein as a "contaminating protein"), more preferably less than about 20% of non-TSRX protein, still more preferably less than about 10% of non-TSRX protein, and most preferably less than about 5% non-TSRX protein. When the TSRX protein or biologically active portion thereof is recombinantly produced, it is also preferably substantially free of culture medium, i.e., culture medium represents less than about 20%, more preferably less than about 10%, and most preferably less than about 5% of the volume of the protein preparation.

The language "substantially free of chemical precursors or other chemicals" includes

language "substantially free of chemical precursors or other chemicals" includes preparations of TSRX protein having less than about 30% (by dry weight) of chemical precursors or non-TSRX chemicals, more preferably less than about 20% chemical precursors or non-TSRX chemicals, still more preferably less than about 10% chemical precursors or non-TSRX chemicals, and most preferably less than about 5% chemical precursors or non-TSRX chemicals.

Biologically active portions of a TSRX protein include peptides comprising amino acid sequences sufficiently homologous to or derived from the amino acid sequence of the TSRX protein, e.g., the amino acid sequence shown in SEQ ID NO: 2 and 4 that include fewer amino acids than the full length TSRX proteins, and exhibit at least one activity of a TSRX protein. Typically, biologically active portions comprise a domain or motif with at least one activity of the TSRX protein. A biologically active portion of a TSRX protein can be a polypeptide which is, for example, 10, 25, 50, 100 or more amino acids in length.

A biologically active portion of a TSRX protein of the present invention may contain at least one of the above-identified domains conserved between the TSRX proteins, e.g., TSR modules. Moreover, other biologically active portions, in which other regions of the protein are deleted, can be prepared by recombinant techniques and evaluated for one or more of the functional activities of a native TSRX protein.

In an embodiment, the TSRX protein has an amino acid sequence shown in SEQ ID NO: 2 and 4. In other embodiments, the TSRX protein is substantially homologous to SEQ ID NO: 2 and 4 and retains the functional activity of the protein of SEQ ID NO: 2 and 4, yet differs in amino acid sequence due to natural allelic variation or mutagenesis, as described in detail below. Accordingly, in another embodiment, the TSRX protein is a protein that comprises an amino acid sequence at least about 45% homologous to the amino acid sequence of SEQ ID NO: 2 and 4 and retains the functional activity of the TSRX proteins of SEQ ID NO: 2 and 4.

Determining homology between two or more sequence

To determine the percent homology of two amino acid sequences or of two nucleic acids, the sequences are aligned for optimal comparison purposes (e.g., gaps can be introduced

positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are homologous at that position (*i.e.* as used herein amino acid or nucleic acid "homology" is equivalent to amino acid or nucleic acid "identity").

The nucleic acid sequence homology may be determined as the degree of identity between two sequences. The homology may be determined using computer programs known in the art, such as GAP software provided in the GCG program package. See, *Needleman and Wunsch* 1970 *J Mol Biol* 48: 443-453. Using GCG GAP software with the following settings for nucleic acid sequence comparison: GAP creation penalty of 5.0 and GAP extension penalty of 0.3, the coding region of the analogous nucleic acid sequences referred to above exhibits a degree of identity preferably of at least 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 99%, with the CDS (encoding) part of the DNA sequence shown in SEQ ID NO: 1 and 3.

The term "sequence identity" refers to the degree to which two polynucleotide or polypeptide sequences are identical on a residue-by-residue basis over a particular region of comparison. The term "percentage of sequence identity" is calculated by comparing two optimally aligned sequences over that region of comparison, determining the number of positions at which the identical nucleic acid base (*e.g.*, A, T, C, G, U, or I, in the case of nucleic acids) occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the region of comparison (*i.e.*, the window size), and multiplying the result by 100 to yield the percentage of sequence identity. The term "substantial identity" as used herein denotes a characteristic of a polynucleotide sequence, wherein the polynucleotide comprises a sequence that has at least 80 percent sequence identity, preferably at least 85 percent identity and often 90 to 95 percent sequence identity, more usually at least 99 percent sequence identity as compared to a reference sequence over a comparison region. The term "percentage of positive residues" is calculated by comparing two optimally aligned sequences over that region of comparison, determining the number of positions at which the identical and conservative amino acid substitutions, as defined above, occur in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the region of comparison (*i.e.*, the window size), and multiplying the result by 100 to yield the

Chimeric and fusion proteins

The invention also provides TSRX chimeric or fusion proteins. As used herein, a TSRX "chimeric protein" or "fusion protein" comprises a TSRX polypeptide operatively linked to a non-TSRX polypeptide. An "TSRX polypeptide" refers to a polypeptide having an amino acid sequence corresponding to TSRX, whereas a "non-TSRX polypeptide" refers to a polypeptide having an amino acid sequence corresponding to a protein that is not substantially homologous to the TSRX protein, *e.g.*, a protein that is different from the TSRX protein and that is derived from the same or a different organism. Within a TSRX fusion protein the TSRX polypeptide can correspond to all or a portion of a TSRX protein. In one embodiment, a TSRX fusion protein comprises at least one biologically active portion of a TSRX protein. In another embodiment, a TSRX fusion protein comprises at least two biologically active portions of a TSRX protein. Within the fusion protein, the term "operatively linked" is intended to indicate that the TSRX polypeptide and the non-TSRX polypeptide are fused in-frame to each other. The non-TSRX polypeptide can be fused to the N-terminus or C-terminus of the TSRX polypeptide.

For example, in one embodiment a TSRX fusion protein comprises a TSRX polypeptide operably linked to the extracellular domain of a second protein. Such fusion proteins can be further utilized in screening assays for compounds that modulate TSRX activity (such assays are described in detail below).

In another embodiment, the fusion protein is a GST-TSRX fusion protein in which the TSRX sequences are fused to the C-terminus of the GST (i.e., glutathione S-transferase) sequences. Such fusion proteins can facilitate the purification of recombinant TSRX.

In another embodiment, the fusion protein is a TSRX-immunoglobulin fusion protein in which the TSRX sequences comprising one or more domains are fused to sequences derived from a member of the immunoglobulin protein family. The TSRX-immunoglobulin fusion proteins of the invention can be incorporated into pharmaceutical compositions and administered to a subject to inhibit an interaction between a TSRX ligand and a TSRX protein on the surface of a cell, to thereby suppress TSRX-mediated signal transduction *in vivo*. In one nonlimiting example, a contemplated TSRX ligand of the invention is the TSRX receptor. The TSRX-immunoglobulin fusion proteins can be used to affect the bioavailability of a

modulating (e.g., promoting or inhibiting) cell survival. Moreover, the TSRX-immunoglobulin fusion proteins of the invention can be used as immunogens to produce anti-TSRX antibodies in a subject, to purify TSRX ligands, and in screening assays to identify molecules that inhibit the interaction of TSRX with a TSRX ligand.

A TSRX chimeric or fusion protein of the invention can be produced by standard recombinant DNA techniques. For example, DNA fragments coding for the different polypeptide sequences are ligated together in-frame in accordance with conventional techniques, *e.g.*, by employing blunt-ended or stagger-ended termini for ligation, restriction enzyme digestion to provide for appropriate termini, filling-in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and enzymatic ligation. In another embodiment, the fusion gene can be synthesized by conventional techniques including automated DNA synthesizers. Alternatively, PCR amplification of gene fragments can be carried out using anchor primers that give rise to complementary overhangs between two consecutive gene fragments that can subsequently be annealed and reamplified to generate a chimeric gene sequence (see, for example, Ausubel et al. (eds.) CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, 1992). Moreover, many expression vectors are commercially available that already encode a fusion moiety (*e.g.*, a GST polypeptide). A TSRX-encoding nucleic acid can be cloned into such an expression vector such that the fusion moiety is linked in-frame to the TSRX protein.

TSRX agonists and antagonists

The present invention also pertains to variants of the TSRX proteins that function as either TSRX agonists (mimetics) or as TSRX antagonists. Variants of the TSRX protein can be generated by mutagenesis, *e.g.*, discrete point mutation or truncation of the TSRX protein. An agonist of the TSRX protein can retain substantially the same, or a subset of, the biological activities of the naturally occurring form of the TSRX protein. An antagonist of the TSRX protein can inhibit one or more of the activities of the naturally occurring form of the TSRX protein by, for example, competitively binding to a downstream or upstream member of a cellular signaling cascade which includes the TSRX protein. Thus, specific biological effects can be elicited by treatment with a variant of limited function. In one embodiment, treatment

form of the protein has fewer side effects in a subject relative to treatment with the naturally occurring form of the TSRX proteins.

Variants of the TSRX protein that function as either TSRX agonists (mimetics) or as TSRX antagonists can be identified by screening combinatorial libraries of mutants, e.g., truncation mutants, of the TSRX protein for TSRX protein agonist or antagonist activity. In one embodiment, a variegated library of TSRX variants is generated by combinatorial mutagenesis at the nucleic acid level and is encoded by a variegated gene library. A variegated library of TSRX variants can be produced by, for example, enzymatically ligating a mixture of synthetic oligonucleotides into gene sequences such that a degenerate set of potential TSRX sequences is expressible as individual polypeptides, or alternatively, as a set of larger fusion proteins (e.g., for phage display) containing the set of TSRX sequences therein. There are a variety of methods which can be used to produce libraries of potential TSRX variants from a degenerate oligonucleotide sequence. Chemical synthesis of a degenerate gene sequence can be performed in an automatic DNA synthesizer, and the synthetic gene then ligated into an appropriate expression vector. Use of a degenerate set of genes allows for the provision, in one mixture, of all of the sequences encoding the desired set of potential TSRX sequences. Methods for synthesizing degenerate oligonucleotides are known in the art (see, e.g., Narang (1983) *Tetrahedron* 39:3; Itakura *et al.* (1984) *Annu Rev Biochem* 53:323; Itakura *et al.* (1984) *Science* 198:1056; Ike *et al.* (1983) *Nucl Acid Res* 11:477.

Polypeptide libraries

In addition, libraries of fragments of the TSRX protein coding sequence can be used to generate a variegated population of TSRX fragments for screening and subsequent selection of variants of a TSRX protein. In one embodiment, a library of coding sequence fragments can be generated by treating a double stranded PCR fragment of a TSRX coding sequence with a nuclease under conditions wherein nicking occurs only about once per molecule, denaturing the double stranded DNA, renaturing the DNA to form double stranded DNA that can include sense/antisense pairs from different nicked products, removing single stranded portions from reformed duplexes by treatment with S1 nuclease, and ligating the resulting fragment library [illegible] this manner, an expression library can be derived which encodes

Several techniques are known in the art for screening gene products of combinatorial libraries made by point mutations or truncation, and for screening cDNA libraries for gene products having a selected property. Such techniques are adaptable for rapid screening of the gene libraries generated by the combinatorial mutagenesis of TSRX proteins. The most widely used techniques, which are amenable to high throughput analysis, for screening large gene libraries typically include cloning the gene library into replicable expression vectors, transforming appropriate cells with the resulting library of vectors, and expressing the combinatorial genes under conditions in which detection of a desired activity facilitates isolation of the vector encoding the gene whose product was detected. Recrusive ensemble mutagenesis (REM), a new technique that enhances the frequency of functional mutants in the libraries, can be used in combination with the screening assays to identify TSRX variants (Arkin and Yourvan (1992) PNAS 89:7811-7815; Delgrave *et al.* (1993) Protein Engineering 6:327-331).

TSRX Antibodies

Also included in the invention are antibodies to TSRX proteins, or fragments of TSRX proteins. The term "antibody" as used herein refers to immunoglobulin molecules and immunologically active portions of immunoglobulin (Ig) molecules, i.e., molecules that contain an antigen binding site that specifically binds (immunoreacts with) an antigen. Such antibodies include, but are not limited to, polyclonal, monoclonal, chimeric, single chain, F_{ab}, $F_{ab'}$ and $F_{(ab')_2}$ fragments, and an F_{ab} expression library. In general, an antibody molecule obtained from humans relates to any of the classes IgG, IgM, IgA, IgE and IgD, which differ from one another by the nature of the heavy chain present in the molecule. Certain classes have subclasses as well, such as IgG1, IgG2, and others. Furthermore, in humans, the light chain may be a kappa chain or a lambda chain. Reference herein to antibodies includes a reference to all such classes, subclasses and types of human antibody species.

An isolated TSRX-related protein of the invention may be intended to serve as an antigen, or a portion or fragment thereof, and additionally can be used as an immunogen to generate antibodies that immunospecifically bind the antigen, using standard techniques for

amino acid sequence of the full length protein, such as an amino acid sequence shown in SEQ ID NO: 2 and 4, and encompasses an epitope thereof such that an antibody raised against the peptide forms a specific immune complex with the full length protein or with any fragment that contains the epitope. Preferably, the antigenic peptide comprises at least 10 amino acid residues, or at least 15 amino acid residues, or at least 20 amino acid residues, or at least 30 amino acid residues. Preferred epitopes encompassed by the antigenic peptide are regions of the protein that are located on its surface; commonly these are hydrophilic regions.

In certain embodiments of the invention, at least one epitope encompassed by the antigenic peptide is a region of TSRX-related protein that is located on the surface of the protein, e.g., a hydrophilic region. A hydrophobicity analysis of the human TSRX-related protein sequence will indicate which regions of a TSRX-related protein are particularly hydrophilic and, therefore, are likely to encode surface residues useful for targeting antibody production. As a means for targeting antibody production, hydropathy plots showing regions of hydrophilicity and hydrophobicity may be generated by any method well known in the art, including, for example, the Kyte Doolittle or the Hopp Woods methods, either with or without Fourier transformation. See, e.g., Hopp and Woods, 1981, *Proc. Nat. Acad. Sci. USA* 78: 3824-3828; Kyte and Doolittle 1982, *J. Mol. Biol.* 157: 105-142, each of which is incorporated herein by reference in its entirety. Antibodies that are specific for one or more domains within an antigenic protein, or derivatives, fragments, analogs or homologs thereof, are also provided herein.

A protein of the invention, or a derivative, fragment, analog, homolog or ortholog thereof, may be utilized as an immunogen in the generation of antibodies that immunospecifically bind these protein components.

Various procedures known within the art may be used for the production of polyclonal or monoclonal antibodies directed against a protein of the invention, or against derivatives, fragments, analogs homologs or orthologs thereof (see, for example, Antibodies, A Laboratory Manual, Harlow E. and Lane D, 1988, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, incorporated herein by reference). Some of these antibodies are discussed below.

For the production of polyclonal antibodies, various suitable host animals (e.g., rabbit, goat, mouse or other mammal) may be immunized by one or more injections with the native protein, a synthetic variant thereof, or a derivative of the foregoing. An appropriate immunogenic preparation can contain, for example, the naturally occurring immunogenic protein, a chemically synthesized polypeptide representing the immunogenic protein, or a recombinantly expressed immunogenic protein. Furthermore, the protein may be conjugated to a second protein known to be immunogenic in the mammal being immunized. Examples of such immunogenic proteins include but are not limited to keyhole limpet hemocyanin, serum albumin, bovine thyroglobulin, and soybean trypsin inhibitor. The preparation can further include an adjuvant. Various adjuvants used to increase the immunological response include, but are not limited to, Freund's (complete and incomplete), mineral gels (e.g., aluminum hydroxide), surface active substances (e.g., lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, dinitrophenol, etc.), adjuvants usable in humans such as Bacille Calmette-Guerin and Corynebacterium parvum, or similar immunostimulatory agents. Additional examples of adjuvants which can be employed include MPL-TDM adjuvant (monophosphoryl Lipid A, synthetic trehalose dicorynomycolate).

The polyclonal antibody molecules directed against the immunogenic protein can be isolated from the mammal (e.g., from the blood) and further purified by well known techniques, such as affinity chromatography using protein A or protein G, which provide primarily the IgG fraction of immune serum. Subsequently, or alternatively, the specific antigen which is the target of the immunoglobulin sought, or an epitope thereof, may be immobilized on a column to purify the immune specific antibody by immunoaffinity chromatography. Purification of immunoglobulins is discussed, for example, by D. Wilkinson (The Scientist, published by The Scientist, Inc., Philadelphia PA, Vol. 14, No. 8 (April 17, 2000), pp. 25-28).

Monoclonal Antibodies

The term "monoclonal antibody" (MAb) or "monoclonal antibody composition" as used herein refers to a population of antibody molecules that contain only one molecular

the monoclonal antibody are identical in all the molecules of the population. MAbs thus contain an antigen binding site capable of immunoreacting with a particular epitope of the antigen characterized by a unique binding affinity for it.

Monoclonal antibodies can be prepared using hybridoma methods, such as those described by Kohler and Milstein, Nature, 256:495 (1975). In a hybridoma method, a mouse, hamster, or other appropriate host animal, is typically immunized with an immunizing agent to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the immunizing agent. Alternatively, the lymphocytes can be immunized in vitro.

The immunizing agent will typically include the protein antigen, a fragment thereof or a fusion protein thereof. Generally, either peripheral blood lymphocytes are used if cells of human origin are desired, or spleen cells or lymph node cells are used if non-human mammalian sources are desired. The lymphocytes are then fused with an immortalized cell line using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell (Goding, Monoclonal Antibodies: Principles and Practice, Academic Press, (1986) pp. 59-103). Immortalized cell lines are usually transformed mammalian cells, particularly myeloma cells of rodent, bovine and human origin. Usually, rat or mouse myeloma cell lines are employed. The hybridoma cells can be cultured in a suitable culture medium that preferably contains one or more substances that inhibit the growth or survival of the unfused, immortalized cells. For example, if the parental cells lack the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT or HPRT), the culture medium for the hybridomas typically will include hypoxanthine, aminopterin, and thymidine ("HAT medium"), which substances prevent the growth of HGPRT-deficient cells.

Preferred immortalized cell lines are those that fuse efficiently, support stable high level expression of antibody by the selected antibody-producing cells, and are sensitive to a medium, such as HAT medium. More preferred immortalized cell lines are murine myeloma lines, which can be obtained, for instance, from the Salk Institute Cell Distribution Center, San Diego, California and the American Type Culture Collection, Manassas, Virginia. Human myeloma and mouse-human heteromyeloma cell lines also have been described for the production of human monoclonal antibodies (Kozbor, J. Immunol., 133:3001 (1984); Brodeur

The culture medium in which the hybridoma cells are cultured can then be assayed for the presence of monoclonal antibodies directed against the antigen. Preferably, the binding specificity of monoclonal antibodies produced by the hybridoma cells is determined by immunoprecipitation or by an in vitro binding assay, such as radioimmunoassay (RIA) or enzyme-linked immunoabsorbent assay (ELISA). Such techniques and assays are known in the art. The binding affinity of the monoclonal antibody can, for example, be determined by the Scatchard analysis of Munson and Pollard, Anal. Biochem., 107:220 (1980). Preferably, antibodies having a high degree of specificity and a high binding affinity for the target antigen are isolated.

After the desired hybridoma cells are identified, the clones can be subcloned by limiting dilution procedures and grown by standard methods. Suitable culture media for this purpose include, for example, Dulbecco's Modified Eagle's Medium and RPMI-1640 medium. Alternatively, the hybridoma cells can be grown in vivo as ascites in a mammal.

The monoclonal antibodies secreted by the subclones can be isolated or purified from the culture medium or ascites fluid by conventional immunoglobulin purification procedures such as, for example, protein A-Sepharose, hydroxylapatite chromatography, gel electrophoresis, dialysis, or affinity chromatography.

The monoclonal antibodies can also be made by recombinant DNA methods, such as those described in U.S. Patent No. 4,816,567. DNA encoding the monoclonal antibodies of the invention can be readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of murine antibodies). The hybridoma cells of the invention serve as a preferred source of such DNA. Once isolated, the DNA can be placed into expression vectors, which are then transfected into host cells such as simian COS cells, Chinese hamster ovary (CHO) cells, or myeloma cells that do not otherwise produce immunoglobulin protein, to obtain the synthesis of monoclonal antibodies in the recombinant host cells. The DNA also can be modified, for example, by substituting the coding sequence for human heavy and light chain constant domains in place of the homologous murine sequences (U.S. Patent No. 4,816,567; Morrison, Nature 368, 812-13 (1994)) or by covalently joining to the

domains of an antibody of the invention, or can be substituted for the variable domains of one antigen-combining site of an antibody of the invention to create a chimeric bivalent antibody.

Humanized Antibodies

The antibodies directed against the protein antigens of the invention can further comprise humanized antibodies or human antibodies. These antibodies are suitable for administration to humans without engendering an immune response by the human against the administered immunoglobulin. Humanized forms of antibodies are chimeric immunoglobulins, immunoglobulin chains or fragments thereof (such as Fv, Fab, Fab', F(ab')$_2$ or other antigen-binding subsequences of antibodies) that are principally comprised of the sequence of a human immunoglobulin, and contain minimal sequence derived from a non-human immunoglobulin. Humanization can be performed following the method of Winter and co-workers (Jones et al., Nature, 321:522-525 (1986); Riechmann et al., Nature, 332:323-327 (1988); Verhoeyen et al., Science, 239:1534-1536 (1988)), by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody. (See also U.S. Patent No. 5,225,539.) In some instances, Fv framework residues of the human immunoglobulin are replaced by corresponding non-human residues. Humanized antibodies can also comprise residues which are found neither in the recipient antibody nor in the imported CDR or framework sequences. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the framework regions are those of a human immunoglobulin consensus sequence. The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin (Jones et al., 1986; Riechmann et al., 1988; and Presta, Curr Op Struct Biol., 2:593-596 (1992)).

Human Antibodies

Fully human antibodies relate to antibody molecules in which essentially the entire

Human monoclonal antibodies can be prepared by the trioma technique; the human B-cell hybridoma technique (see Kozbor, et al., 1983 Immunol Today 4: 72) and the EBV hybridoma technique to produce human monoclonal antibodies (see Cole, et al., 1985 In: MONOCLONAL ANTIBODIES AND CANCER THERAPY, Alan R. Liss, Inc., pp. 77-96). Human monoclonal antibodies may be utilized in the practice of the present invention and may be produced by using human hybridomas (see Cote, et al., 1983. Proc Natl Acad Sci USA 80: 2026-2030) or by transforming human B-cells with Epstein Barr Virus in vitro (see Cole, et al. 1985 In: MONOCLONAL ANTIBODIES AND CANCER THERAPY, Alan R. Liss, Inc., pp. 77-96).

In addition, human antibodies can also be produced using additional techniques, including phage display libraries (Hoogenboom and Winter, J. Mol. Biol., 227:381 (1991); Marks et al., J. Mol. Biol., 222 581 (1991)). Similarly, human antibodies can be made by introducing human immunoglobulin loci into transgenic animals, e.g., mice in which the endogenous immunoglobulin genes have been partially or completely inactivated. Upon challenge, human antibody production is observed, which closely resembles that seen in humans in all respects, including gene rearrangement, assembly, and antibody repertoire. This approach is described, for example, in U.S. Patent Nos. 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425 5,661,016, and in Marks et al. (Bio Technology 10, 779-783 (1992)); Lonberg et al. (Nature 368 856-859 (1994)); Morrison (Nature 368, 812-13 (1994)); Fishwild et al.(Nature Biotechnology 14, 845-51 (1996)); Neuberger (Nature Biotechnology 14, 826 (1996)); and Lonberg and Huszar (Intern. Rev. Immunol. 13 65-93 (1995)).

Human antibodies may additionally be produced using transgenic nonhuman animals which are modified so as to produce fully human antibodies rather than the animal's endogenous antibodies in response to challenge by an antigen. (See PCT publication WO94/02602). The endogenous genes encoding the heavy and light immunoglobulin chains in the nonhuman host have been incapacitated, and active loci encoding human heavy and light chain immunoglobulins are inserted into the host's genome. The human genes are incorporated, for example, using yeast artificial chromosomes containing the requisite human DNA segments. An animal which provides all the desired modifications is then obtained as progeny by crossbreeding intermediate transgenic animals containing fewer than the full

WO 96/34096. This animal produces B cells which secrete fully human immunoglobulins. The antibodies can be obtained directly from the animal after immunization with an immunogen of interest, as, for example, a preparation of a polyclonal antibody, or alternatively from immortalized B cells derived from the animal, such as hybridomas producing monoclonal antibodies. Additionally, the genes encoding the immunoglobulins with human variable regions can be recovered and expressed to obtain the antibodies directly, or can be further modified to obtain analogs of antibodies such as, for example, single chain Fv molecules.

An example of a method of producing a nonhuman host, exemplified as a mouse, lacking expression of an endogenous immunoglobulin heavy chain is disclosed in U.S. Patent No. 5,939,598. It can be obtained by a method including deleting the J segment genes from at least one endogenous heavy chain locus in an embryonic stem cell to prevent rearrangement of the locus and to prevent formation of a transcript of a rearranged immunoglobulin heavy chain locus, the deletion being effected by a targeting vector containing a gene encoding a selectable marker, and producing from the embryonic stem cell a transgenic mouse whose somatic and germ cells contain the gene encoding the selectable marker.

A method for producing an antibody of interest, such as a human antibody, is disclosed in U.S. Patent No. 5,916,771. It includes introducing an expression vector that contains a nucleotide sequence encoding a heavy chain into one mammalian host cell in culture, introducing an expression vector containing a nucleotide sequence encoding a light chain into another mammalian host cell, and fusing the two cells to form a hybrid cell. The hybrid cell expresses an antibody containing the heavy chain and the light chain.

In a further improvement on this procedure, a method for identifying a clinically relevant epitope on an immunogen, and a correlative method for selecting an antibody that binds immunospecifically to the relevant epitope with high affinity, are disclosed in PCT publication WO 99/53049.

F$_v$ Fragments and Single Chain Antibodies

According to the invention, techniques can be adapted for the production of

libraries (see e.g., Huse, et al., 1989 Science 246: 1275-1281) to allow rapid and effective identification of monoclonal F_{ab} fragments with the desired specificity for a protein or derivatives, fragments, analogs or homologs thereof. Antibody fragments that contain the idiotypes to a protein antigen may be produced by techniques known in the art including, but not limited to: (i) an $F_{(ab')2}$ fragment produced by pepsin digestion of an antibody molecule; (ii) an $F_{ab'}$ fragment generated by reducing the disulfide bridges of an $F_{(ab')2}$ fragment; (iii) an F_{ab} fragment generated by the treatment of the antibody molecule with papain and a reducing agent and (iv) F_v fragments.

Bispecific Antibodies

Bispecific antibodies are monoclonal, preferably human or humanized, antibodies that have binding specificities for at least two different antigens. In the present case, one of the binding specificities is for an antigenic protein of the invention. The second binding target is any other antigen, and advantageously is a cell-surface protein or receptor or receptor subunit.

Methods for making bispecific antibodies are known in the art. Traditionally, the recombinant production of bispecific antibodies is based on the co-expression of two immunoglobulin heavy-chain light-chain pairs, where the two heavy chains have different specificities (Milstein and Cuello, Nature, 305:537-539 (1983)). Because of the random assortment of immunoglobulin heavy and light chains, these hybridomas (quadromas) produce a potential mixture of ten different antibody molecules, of which only one has the correct bispecific structure. The purification of the correct molecule is usually accomplished by affinity chromatography steps. Similar procedures are disclosed in WO 93/08829, published 13 May 1993, and in Traunecker et al., 1991 *EMBO J.*, 10:3655-3659.

Antibody variable domains with the desired binding specificities (antibody-antigen combining sites) can be fused to immunoglobulin constant domain sequences. The fusion preferably is with an immunoglobulin heavy-chain constant domain, comprising at least part of the hinge, CH2, and CH3 regions. It is preferred to have the first heavy-chain constant region (CH1) containing the site necessary for light-chain binding present in at least one of the

transfected into a suitable host organism. For further details of generating bispecific antibodies see, for example, Suresh et al., Methods in Enzymology, 121:210 (1986).

According to another approach described in WO 96/27011, the interface between a pair of antibody molecules can be engineered to maximize the percentage of heterodimers which are recovered from recombinant cell culture. The preferred interface comprises at least a part of the CH3 region of an antibody constant domain. In this method, one or more small amino acid side chains from the interface of the first antibody molecule are replaced with larger side chains (e.g. tyrosine or tryptophan). Compensatory "cavities" of identical or similar size to the large side chain(s) are created on the interface of the second antibody molecule by replacing large amino acid side chains with smaller ones (e.g. alanine or threonine). This provides a mechanism for increasing the yield of the heterodimer over other unwanted end-products such as homodimers.

Bispecific antibodies can be prepared as full length antibodies or antibody fragments (e.g. $F(ab')_2$ bispecific antibodies). Techniques for generating bispecific antibodies from antibody fragments have been described in the literature. For example, bispecific antibodies can be prepared using chemical linkage. Brennan et al., Science 229:81 (1985) describe a procedure wherein intact antibodies are proteolytically cleaved to generate $F(ab')_2$ fragments. These fragments are reduced in the presence of the dithiol complexing agent sodium arsenite to stabilize vicinal dithiols and prevent intermolecular disulfide formation. The Fab' fragments generated are then converted to thionitrobenzoate (TNB) derivatives. One of the Fab'-TNB derivatives is then reconverted to the Fab'-thiol by reduction with mercaptoethylamine and is mixed with an equimolar amount of the other Fab'-TNB derivative to form the bispecific antibody. The bispecific antibodies produced can be used as agents for the selective immobilization of enzymes.

Additionally, Fab' fragments can be directly recovered from E. coli and chemically coupled to form bispecific antibodies. Shalaby et al., J. Exp. Med. 175:217-225 (1992) describe the production of a fully humanized bispecific antibody $F(ab')_2$ molecule. Each Fab' fragment was separately secreted from E. coli and subjected to directed chemical coupling in vitro to form the bispecific antibody. The bispecific antibody thus formed was able to bind to

Various techniques for making and isolating bispecific antibody fragments directly from recombinant cell culture have also been described. For example, bispecific antibodies have been produced using leucine zippers. Kostelny et al., J. Immunol. 148(5):1547-1553 (1992). The leucine zipper peptides from the Fos and Jun proteins were linked to the Fab' portions of two different antibodies by gene fusion. The antibody homodimers were reduced at the hinge region to form monomers and then re-oxidized to form the antibody heterodimers. This method can also be utilized for the production of antibody homodimers. The "diabody" technology described by Hollinger et al., Proc. Natl. Acad. Sci. USA 90:6444-6448 (1993) has provided an alternative mechanism for making bispecific antibody fragments. The fragments comprise a heavy-chain variable domain (V_H) connected to a light-chain variable domain (V_L) by a linker which is too short to allow pairing between the two domains on the same chain. Accordingly, the V_H and V_L domains of one fragment are forced to pair with the complementary V_L and V_H domains of another fragment, thereby forming two antigen-binding sites. Another strategy for making bispecific antibody fragments by the use of single-chain Fv (sFv) dimers has also been reported. See, Gruber et al., J. Immunol. 152:5368 (1994).

Antibodies with more than two valencies are contemplated. For example, trispecific antibodies can be prepared. Tutt et al., J. Immunol. 147:60 (1991).

Exemplary bispecific antibodies can bind to two different epitopes, at least one of which originates in the protein antigen of the invention. Alternatively, an anti-antigenic arm of an immunoglobulin molecule can be combined with an arm which binds to a triggering molecule on a leukocyte such as a T-cell receptor molecule (e.g. CD2, CD3, CD28, or B7), or Fc receptors for IgG (FcγR), such as FcγRI (CD64), FcγRII (CD32) and FcγRIII (CD16) so as to focus cellular defense mechanisms to the cell expressing the particular antigen. Bispecific antibodies can also be used to direct cytotoxic agents to cells which express a particular antigen. These antibodies possess an antigen-binding arm and an arm which binds a cytotoxic agent or a radionuclide chelator, such as EOTUBE, DPTA, DOTA, or TETA. Another bispecific antibody of interest binds the protein antigen described herein and further binds tissue factor (TF).

Heteroconjugate antibodies are also within the scope of the present invention. Heteroconjugate antibodies are composed of two covalently joined antibodies. Such antibodies have, for example, been proposed to target immune system cells to unwanted cells (U.S. Patent No. 4,676,980), and for treatment of HIV infection (WO 91/00360; WO 92/200373; EP 03089). It is contemplated that the antibodies can be prepared in vitro using known methods in synthetic protein chemistry, including those involving crosslinking agents. For example, immunotoxins can be constructed using a disulfide exchange reaction or by forming a thioether bond. Examples of suitable reagents for this purpose include iminothiolate and methyl-4-mercaptobutyrimidate and those disclosed, for example, in U.S. Patent No. 4,676,980.

Effector Function Engineering

It can be desirable to modify the antibody of the invention with respect to effector function, so as to enhance, e.g., the effectiveness of the antibody in treating cancer. For example, cysteine residue(s) can be introduced into the Fc region, thereby allowing interchain disulfide bond formation in this region. The homodimeric antibody thus generated can have improved internalization capability and or increased complement-mediated cell killing and antibody-dependent cellular cytotoxicity (ADCC). See Caron et al., J. Exp Med., 176: 1191-1195 (1992) and Shopes, J. Immunol., 148: 2918-2922 (1992). Homodimeric antibodies with enhanced anti-tumor activity can also be prepared using heterobifunctional cross-linkers as described in Wolff et al. Cancer Research, 53: 2560-2565 (1993). Alternatively, an antibody can be engineered that has dual Fc regions and can thereby have enhanced complement lysis and ADCC capabilities. See Stevenson et al., Anti-Cancer Drug Design, 3: 219-230 (1989).

Immunoconjugates

The invention also pertains to immunoconjugates comprising an antibody conjugated to a cytotoxic agent such as a chemotherapeutic agent, toxin (e.g., an enzymatically active toxin of bacterial, fungal, plant, or animal origin, or fragments thereof), or a radioactive

Chemotherapeutic agents useful in the generation of such immunoconjugates have been described above. Enzymatically active toxins and fragments thereof that can be used include diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from Pseudomonas aeruginosa), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, Aleurites fordii proteins, dianthin proteins, Phytolaca americana proteins (PAPI, PAPII, and PAP-S), momordica charantia inhibitor, curcin, crotin, sapaonaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin, and the tricothecenes. A variety of radionuclides are available for the production of radioconjugated antibodies. Examples include ^{212}Bi, ^{131}I, ^{111}In, ^{90}Y, and ^{186}Re.

Conjugates of the antibody and cytotoxic agent are made using a variety of bifunctional protein-coupling agents such as N-succinimidyl-3-(2-pyridyldithiol) propionate (SPDP), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCL), active esters (such as disuccinimidyl suberate), aldehydes (such as glutareldehyde), bis-azido compounds (such as bis (p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as tolyene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). For example, a ricin immunotoxin can be prepared as described in Vitetta et al., Science, 238: 1098 (1987). Carbon-14-labeled 1-isothiocyanatobenzyl-3-methyldiethylene triaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to the antibody. See WO94 11026.

In another embodiment, the antibody can be conjugated to a "receptor" (such streptavidin) for utilization in tumor pretargeting wherein the antibody-receptor conjugate is administered to the patient, followed by removal of unbound conjugate from the circulation using a clearing agent and then administration of a "ligand" (e.g., avidin) that is in turn conjugated to a cytotoxic agent.

TSRX Recombinant Expression Vectors and Host Cells

Another aspect of the invention pertains to vectors, preferably expression vectors,

of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments can be ligated. Another type of vector is a viral vector, wherein additional DNA segments can be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (e.g., bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (e.g., non-episomal mammalian vectors) are integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively-linked. Such vectors are referred to herein as "expression vectors". In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" can be used interchangeably as the plasmid is the most commonly used form of vector. However, the invention is intended to include such other forms of expression vectors, such as viral vectors (e.g., replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions.

The recombinant expression vectors of the invention comprise a nucleic acid of the invention in a form suitable for expression of the nucleic acid in a host cell, which means that the recombinant expression vectors include one or more regulatory sequences, selected on the basis of the host cells to be used for expression, that is operatively-linked to the nucleic acid sequence to be expressed. Within a recombinant expression vector, "operably-linked" is intended to mean that the nucleotide sequence of interest is linked to the regulatory sequence(s) in a manner that allows for expression of the nucleotide sequence (e.g., in an *in vitro* transcription/translation system or in a host cell when the vector is introduced into the host cell).

The term "regulatory sequence" is intended to includes promoters, enhancers and other expression control elements (e.g., polyadenylation signals). Such regulatory sequences are described, for example, in Goeddel, GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990). Regulatory sequences include those that direct constitutive expression of a nucleotide sequence in many types of host cell

design of the expression vector can depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, etc. The expression vectors of the invention can be introduced into host cells to thereby produce proteins or peptides, including fusion proteins or peptides, encoded by nucleic acids as described herein (e.g., TSRX proteins, mutant forms of TSRX proteins, fusion proteins, etc.).

The recombinant expression vectors of the invention can be designed for expression of TSRX proteins in prokaryotic or eukaryotic cells. For example, TSRX proteins can be expressed in bacterial cells such as *Escherichia coli*, insect cells (using baculovirus expression vectors) yeast cells or mammalian cells. Suitable host cells are discussed further in Goeddel, GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990). Alternatively, the recombinant expression vector can be transcribed and translated *in vitro*, for example using T7 promoter regulatory sequences and T7 polymerase.

Expression of proteins in prokaryotes is most often carried out in *Escherichia coli* with vectors containing constitutive or inducible promoters directing the expression of either fusion or non-fusion proteins. Fusion vectors add a number of amino acids to a protein encoded therein, usually to the amino terminus of the recombinant protein. Such fusion vectors typically serve three purposes: (*i*) to increase expression of recombinant protein; (*ii*) to increase the solubility of the recombinant protein; and (*iii*) to aid in the purification of the recombinant protein by acting as a ligand in affinity purification. Often, in fusion expression vectors, a proteolytic cleavage site is introduced at the junction of the fusion moiety and the recombinant protein to enable separation of the recombinant protein from the fusion moiety subsequent to purification of the fusion protein. Such enzymes, and their cognate recognition sequences, include Factor Xa, thrombin and enterokinase. Typical fusion expression vectors include pGEX (Pharmacia Biotech Inc; Smith and Johnson, 1988, *Gene* 67: 31-40), pMAL (New England Biolabs, Beverly, Mass.) and pRIT5 (Pharmacia, Piscataway, N.J.) that fuse glutathione S-transferase (GST), maltose E binding protein, or protein A, respectively, to the target recombinant protein.

Examples of suitable inducible non-fusion *E. coli* expression vectors include pTrc (Amrann *et al.*, (1988) *Gene* 69:301-315) and pET 11d (Studier *et al.*, GENE EXPRESSION

One strategy to maximize recombinant protein expression in *E. coli* is to express the protein in a host bacteria with an impaired capacity to proteolytically cleave the recombinant protein. See e.g., Gottesman, GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990) 119-128. Another strategy is to alter the nucleic acid sequence of the nucleic acid to be inserted into an expression vector so that the individual codons for each amino acid are those preferentially utilized in *E. coli* (see e.g. Wada, *et al.*, 1992. *Nucl. Acids Res.* 20: 2111-2118). Such alteration of nucleic acid sequences of the invention can be carried out by standard DNA synthesis techniques.

In another embodiment, the TSRX expression vector is a yeast expression vector. Examples of vectors for expression in yeast *Saccharomyces cerivisiae* include pYepSec1 (Baldari, *et al.*, 1987. *EMBO J.* 6: 229-234), pMFa (Kurjan and Herskowitz, 1982. *Cell* 30: 933-943), pJRY88 (Schultz *et al.*, 1987. *Gene* 54: 113-123), pYES2 (Invitrogen Corporation, San Diego, Calif.), and picZ (InVitrogen Corp, San Diego, Calif.).

Alternatively, TSRX can be expressed in insect cells using baculovirus expression vectors. Baculovirus vectors available for expression of proteins in cultured insect cells (e.g., SF9 cells) include the pAc series (Smith, *et al.*, 1983. *Mol. Cell. Biol.* 3: 2156-2165) and the pVL series (Lucklow and Summers, 1989. *Virology* 170: 31-39).

In yet another embodiment, a nucleic acid of the invention is expressed in mammalian cells using a mammalian expression vector. Examples of mammalian expression vectors include pCDM8 (Seed, 1987. *Nature* 329: 840) and pMT2PC (Kaufman, *et al.*, 1987. *EMBO J.* 6: 187-195). When used in mammalian cells, the expression vector's control functions are often provided by viral regulatory elements. For example, commonly used promoters are derived from polyoma, adenovirus 2, cytomegalovirus, and simian virus 40. For other suitable expression systems for both prokaryotic and eukaryotic cells see, e.g., Chapters 16 and 17 of Sambrook, *et al.*, MOLECULAR CLONING: A LABORATORY MANUAL, 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989.

In another embodiment, the recombinant mammalian expression vector is capable of directing expression of the nucleic acid preferentially in a particular cell type (e.g., tissue-specific regulatory elements are used to express the nucleic acid). Tissue-specific

268-277), lymphoid-specific promoters (Calame and Eaton, 1988, *Adv. Immunol.* 43: 235-275), in particular promoters of T cell receptors (Winoto and Baltimore, 1989, *EMBO J.* 8: 729-733) and immunoglobulins (Banerji, *et al.*, 1983, *Cell* 33: 729-740; Queen and Baltimore, 1983, *Cell* 33: 741-748), neuron-specific promoters (e.g., the neurofilament promoter; Byrne and Ruddle, 1989, *Proc. Natl. Acad. Sci. USA* 86: 5473-5477), pancreas-specific promoters (Edlund, *et al.*, 1985, *Science* 230: 912-916), and mammary gland-specific promoters (e.g., milk whey promoter; U.S. Pat. No. 4,873,316 and European Application Publication No. 264,166). Developmentally-regulated promoters are also encompassed, e.g., the murine hox promoters (Kessel and Gruss, 1990, *Science* 249: 374-379) and the α-fetoprotein promoter (Campes and Tilghman, 1989, *Genes Dev.* 3: 537-546).

The invention further provides a recombinant expression vector comprising a DNA molecule of the invention cloned into the expression vector in an antisense orientation. That is, the DNA molecule is operatively-linked to a regulatory sequence in a manner that allows for expression (by transcription of the DNA molecule) of an RNA molecule that is antisense to TSRX mRNA. Regulatory sequences operatively linked to a nucleic acid cloned in the antisense orientation can be chosen that direct the continuous expression of the antisense RNA molecule in a variety of cell types, for instance viral promoters and or enhancers, or regulatory sequences can be chosen that direct constitutive, tissue specific or cell type specific expression of antisense RNA. The antisense expression vector can be in the form of a recombinant plasmid, phagemid or attenuated virus in which antisense nucleic acids are produced under the control of a high efficiency regulatory region, the activity of which can be determined by the cell type into which the vector is introduced. For a discussion of the regulation of gene expression using antisense genes see, e.g., Weintraub, *et al.*, "Antisense RNA as a molecular tool for genetic analysis," *Reviews-Trends in Genetics*, Vol. 1(1) 1986.

Another aspect of the invention pertains to host cells into which a recombinant expression vector of the invention has been introduced. The terms "host cell" and "recombinant host cell" are used interchangeably herein. It is understood that such terms refer not only to the particular subject cell but also to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to either

A host cell can be any prokaryotic or eukaryotic cell. For example, TSRX protein can be expressed in bacterial cells such as *E. coli*, insect cells, yeast or mammalian cells (such as human, Chinese hamster ovary cells (CHO) or COS cells). Other suitable host cells are known to those skilled in the art.

Vector DNA can be introduced into prokaryotic or eukaryotic cells via conventional transformation or transfection techniques. As used herein, the terms "transformation" and "transfection" are intended to refer to a variety of art-recognized techniques for introducing foreign nucleic acid (e.g., DNA) into a host cell, including calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, lipofection, or electroporation. Suitable methods for transforming or transfecting host cells can be found in Sambrook, *et al.* (MOLECULAR CLONING: A LABORATORY MANUAL, 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989), and other laboratory manuals.

For stable transfection of mammalian cells, it is known that, depending upon the expression vector and transfection technique used, only a small fraction of cells may integrate the foreign DNA into their genome. In order to identify and select these integrants, a gene that encodes a selectable marker (e.g., resistance to antibiotics) is generally introduced into the host cells along with the gene of interest. Various selectable markers include those that confer resistance to drugs, such as G418, hygromycin and methotrexate. Nucleic acid encoding a selectable marker can be introduced into a host cell on the same vector as that encoding TSRX or can be introduced on a separate vector. Cells stably transfected with the introduced nucleic acid can be identified by drug selection (e.g., cells that have incorporated the selectable marker gene will survive, while the other cells die).

A host cell of the invention, such as a prokaryotic or eukaryotic host cell in culture, can be used to produce (i.e., express) TSRX protein. Accordingly, the invention further provides methods for producing TSRX protein using the host cells of the invention. In one embodiment, the method comprises culturing the host cell of invention (into which a recombinant expression vector encoding TSRX protein has been introduced) in a suitable medium such that TSRX protein is produced. In another embodiment, the method further

Transgenic TSRX Animals

The host cells of the invention can also be used to produce non-human transgenic animals. For example, in one embodiment, a host cell of the invention is a fertilized oocyte or an embryonic stem cell into which TSRX protein-coding sequences have been introduced. Such host cells can then be used to create non-human transgenic animals in which exogenous TSRX sequences have been introduced into their genome or homologous recombinant animals in which endogenous TSRX sequences have been altered. Such animals are useful for studying the function and/or activity of TSRX protein and for identifying and/or evaluating modulators of TSRX protein activity. As used herein, a "transgenic animal" is a non-human animal, preferably a mammal, more preferably a rodent such as a rat or mouse, in which one or more of the cells of the animal includes a transgene. Other examples of transgenic animals include non-human primates, sheep, dogs, cows, goats, chickens, amphibians, etc. A transgene is exogenous DNA that is integrated into the genome of a cell from which a transgenic animal develops and that remains in the genome of the mature animal, thereby directing the expression of an encoded gene product in one or more cell types or tissues of the transgenic animal. As used herein, a "homologous recombinant animal" is a non-human animal, preferably a mammal, more preferably a mouse, in which an endogenous TSRX gene has been altered by homologous recombination between the endogenous gene and an exogenous DNA molecule introduced into a cell of the animal, *e.g.*, an embryonic cell of the animal, prior to development of the animal.

A transgenic animal of the invention can be created by introducing TSRX-encoding nucleic acid into the male pronuclei of a fertilized oocyte (*e.g.*, by microinjection, retroviral infection) and allowing the oocyte to develop in a pseudopregnant female foster animal. Sequences including SEQ ID NO: 1 and 3 can be introduced as a transgene into the genome of a non-human animal. Alternatively, a non-human homologue of the human TSRX gene, such as a mouse TSRX gene, can be isolated based on hybridization to the human TSRX cDNA (described further *supra*) and used as a transgene. Intronic sequences and polyadenylation signals can also be included in the transgene to increase the efficiency of expression of the transgene. A tissue-specific regulatory sequence(s) can be operably-linked to the TSRX

mice, have become conventional in the art and are described, for example, in U.S. Patent Nos. 4,736,866, 4,870,009, and 4,873,191; and Hogan, 1986. In MANIPULATING THE MOUSE EMBRYO, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. Similar methods are used for production of other transgenic animals. A transgenic founder animal can be identified based upon the presence of the TSRX transgene in its genome and/or expression of TSRX mRNA in tissues or cells of the animals. A transgenic founder animal can then be used to breed additional animals carrying the transgene. Moreover, transgenic animals carrying a transgene-encoding TSRX protein can further be bred to other transgenic animals carrying other transgenes.

To create a homologous recombinant animal, a vector is prepared which contains at least a portion of a TSRX gene into which a deletion, addition or substitution has been introduced to thereby alter, *e.g.*, functionally disrupt, the TSRX gene. The TSRX gene can be a human gene (*e.g.*, the DNA of SEQ ID NO: 1 and 3), but more preferably, is a non-human homologue of a human TSRX gene. For example, a mouse homologue of human TSRX gene of SEQ ID NO: 1 and 3 can be used to construct a homologous recombination vector suitable for altering an endogenous TSRX gene in the mouse genome. In one embodiment, the vector is designed such that, upon homologous recombination, the endogenous TSRX gene is functionally disrupted (*i.e.*, no longer encodes a functional protein; also referred to as a "knock out" vector).

Alternatively, the vector can be designed such that, upon homologous recombination, the endogenous TSRX gene is mutated or otherwise altered but still encodes functional protein (*e.g.*, the upstream regulatory region can be altered to thereby alter the expression of the endogenous TSRX protein). In the homologous recombination vector, the altered portion of the TSRX gene is flanked at its 5'- and 3'-termini by additional nucleic acid of the TSRX gene to allow for homologous recombination to occur between the exogenous TSRX gene carried by the vector and an endogenous TSRX gene in an embryonic stem cell. The additional flanking TSRX nucleic acid is of sufficient length for successful homologous recombination with the endogenous gene. Typically, several kilobases of flanking DNA (both at the 5'- and 3'-termini) are included in the vector. See, *e.g.*, Thomas *et al.*, 1987. Cell 51: 503 for a

gene has homologously-recombined with the endogenous TSP.X gene are selected. *See, e.g.*, Li *et al.*, 1992, *Cell* 69: 915.

The selected cells are then injected into a blastocyst of an animal (e.g., a mouse) to form aggregation chimeras. *See, e.g.*, Bradley, 1987, in: TERATOCARCINOMAS AND EMBRYONIC STEM CELLS: A PRACTICAL APPROACH, Robertson, ed. IRL, Oxford, pp. 113-152. A chimeric embryo can then be implanted into a suitable pseudopregnant female foster animal and the embryo brought to term. Progeny harboring the homologously-recombined DNA in their germ cells can be used to breed animals in which all cells of the animal contain the homologously-recombined DNA by germline transmission of the transgene. Methods for constructing homologous recombination vectors and homologous recombinant animals are described further in Bradley, 1991, *Curr. Opin. Biotechnol.* 2: 823-829; PCT International Publication Nos.: WO 90/11354; WO 91/01140, WO 92/0968; and WO 93/04169.

In another embodiment, transgenic non-humans animals can be produced that contain selected systems that allow for regulated expression of the transgene. One example of such a system is the cre loxP recombinase system of bacteriophage P1. For a description of the cre loxP recombinase system, *See, e.g.*, Lakso, *et al.*, 1992, *Proc. Natl. Acad. Sci. USA* 89: 6232-6236. Another example of a recombinase system is the FLP recombinase system of *Saccharomyces cerevisiae*. *See*, O'Gorman, *et al.*, 1991, *Science* 251:1351-1355. If a cre/loxP recombinase system is used to regulate expression of the transgene, animals containing transgenes encoding both the Cre recombinase and a selected protein are required. Such animals can be provided through the construction of "double" transgenic animals, *e.g.*, by mating two transgenic animals, one containing a transgene encoding a selected protein and the other containing a transgene encoding a recombinase.

Clones of the non-human transgenic animals described herein can also be produced according to the methods described in Wilmut, *et al.*, 1997, *Nature* 385: 810-813. In brief, a cell (e.g., a somatic cell) from the transgenic animal can be isolated and induced to exit the growth cycle and enter G₀ phase. The quiescent cell can then be fused, *e.g.*, through the use of electrical pulses, to an enucleated oocyte from an animal of the same species from which the quiescent cell is isolated. The reconstructed oocyte is then cultured such that it develops to

offspring borne of this female foster animal will be a clone of the animal from which the cell (e.g., the somatic cell) is isolated.

Pharmaceutical Compositions

The TSRX nucleic acid molecules, TSRX proteins, and anti-TSRX antibodies (also referred to herein as "active compounds") of the invention, and derivatives, fragments, analogs and homologs thereof, can be incorporated into pharmaceutical compositions suitable for administration. Such compositions typically comprise the nucleic acid molecule, protein, or antibody and a pharmaceutically acceptable carrier. As used herein, "pharmaceutically acceptable carrier" is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. Suitable carriers are described in the most recent edition of Remington's Pharmaceutical Sciences, a standard reference text in the field, which is incorporated herein by reference. Preferred examples of such carriers or diluents include, but are not limited to, water, saline, finger's solutions, dextrose solution, and 5% human serum albumin. Liposomes and non-aqueous vehicles such as fixed oils may also be used. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions.

The antibodies disclosed herein can also be formulated as immunoliposomes. Liposomes containing the antibody are prepared by methods known in the art, such as described in Epstein et al., Proc. Natl. Acad. Sci. USA, 82: 3688 (1985); Hwang et al., Proc. Natl. Acad. Sci. USA, 77: 4030 (1980); and U.S. Pat. Nos. 4,485,045 and 4,544,545. Liposomes with enhanced circulation time are disclosed in U.S. Patent No. 5,013,556.

Particularly useful liposomes can be generated by the reverse-phase evaporation method with a lipid composition comprising phosphatidylcholine, cholesterol, and PEG-derivatized phosphatidylethanolamine (PEG-PE). Liposomes are extruded through filters of defined pore size to yield liposomes with the desired diameter. Fab' fragments of the antibody

agent (such as Doxorubicin) is optionally contained within the liposome. See Gabizon et al., J National Cancer Inst., 81(19): 1484 (1989).

A pharmaceutical composition of the invention is formulated to be compatible with its intended route of administration. Examples of routes of administration include parenteral, e.g., intravenous, intradermal, subcutaneous, oral (e.g., inhalation), transdermal (i.e., topical), transmucosal, and rectal administration. Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid (EDTA); buffers such as acetates, citrates or phosphates, and agents for the adjustment of tonicity such as sodium chloride or dextrose. The pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF, Parsippany, N.J.) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringeability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic

composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions can be prepared by incorporating the active compound (e.g., a TSRX protein or anti-TSRX antibody) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, methods of preparation are vacuum drying and freeze-drying that yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

Oral compositions generally include an inert diluent or an edible carrier. They can be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash, wherein the compound in the fluid carrier is applied orally and swished and expectorated or swallowed. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes, a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin, or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

For administration by inhalation, the compounds are delivered in the form of an aerosol spray from pressured container or dispenser which contains a suitable propellant, e.g., a gas such as carbon dioxide, or a nebulizer.

Systemic administration can also be by transmucosal or transdermal means. For

include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the active compounds are formulated into ointments, salves, gels, or creams as generally known in the art.

The compounds can also be prepared in the form of suppositories (e.g., with conventional suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery.

In one embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Patent No. 4,522,811.

It is especially advantageous to formulate oral or parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated, each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and the limitations inherent in the art of compounding such an active compound for the treatment of individuals.

The nucleic acid molecules of the invention can be inserted into vectors and used as [illegible] expression vectors are then introduced into a subject by, for example,

The pharmaceutical preparation of the gene therapy vector can include the gene therapy vector in an acceptable diluent, or can comprise a slow release matrix in which the gene delivery vehicle is imbedded. Alternatively, where the complete gene delivery vector can be produced intact from recombinant cells, e.g., retroviral vectors, the pharmaceutical preparation can include one or more cells that produce the gene delivery system.

Antibodies specifically binding a protein of the invention, as well as other molecules identified by the screening assays disclosed herein, can be administered for the treatment of various disorders in the form of pharmaceutical compositions. Principles and considerations involved in preparing such compositions, as well as guidance in the choice of components are provided, for example, in Remington : The Science And Practice Of Pharmacy 19th ed (Alfonso R. Gennaro, et al., editors) Mack Pub. Co., Easton, Pa. : 1995; Drug Absorption Enhancement : Concepts, Possibilities, Limitations, And Trends, Harwood Academic Publishers, Langhorne, Pa., 1994; and Peptide And Protein Drug Delivery (Advances In Parenteral Sciences, Vol. 4), 1991, M. Dekker, New York. If the antigenic protein is intracellular and whole antibodies are used as inhibitors, internalizing antibodies are preferred. However, liposomes can also be used to deliver the antibody, or an antibody fragment, into cells. Where antibody fragments are used, the smallest inhibitory fragment that specifically binds to the binding domain of the target protein is preferred. For example, based upon the variable-region sequences of an antibody, peptide molecules can be designed that retain the ability to bind the target protein sequence. Such peptides can be synthesized chemically and or produced by recombinant DNA technology. See, e.g., Marasco *et al.*, 1993 *Proc. Natl. Acad. Sci. U.S.A.* 90: 7889-7893. The formulation herein can also contain more than one active compound as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other. Alternatively, or in addition, the composition can comprise an agent that enhances its function, such as, for example, a cytotoxic agent, cytokine, chemotherapeutic agent, or growth-inhibitory agent. Such molecules are suitably present in combination in amounts that are effective for the purpose intended. The active ingredients can also be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example,

albumin microspheres, microemulsions, nano-particles, and nanocapsules) or in macroemulsions.

The formulations to be used for in vivo administration must be sterile. This is readily accomplished by filtration through sterile filtration membranes.

Sustained-release preparations can be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form of shaped articles, e.g., films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate), or poly(vinylalcohol)), polylactides (U.S. Pat. No. 3,773,919), copolymers of L-glutamic acid and ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOT™ (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(-)-3-hydroxybutyric acid. While polymers such as ethylene-vinyl acetate and lactic acid-glycolic acid enable release of molecules for over 100 days, certain hydrogels release proteins for shorter time periods.

The pharmaceutical compositions can be included in a container, pack, or dispenser together with instructions for administration.

Screening and Detection Methods

The isolated nucleic acid molecules of the invention can be used to express TSRX protein (e.g., via a recombinant expression vector in a host cell in gene therapy applications), to detect TSRX mRNA (e.g., in a biological sample) or a genetic lesion in a TSRX gene, and to modulate TSRX activity, as described further below. In addition, the TSRX proteins can be used to screen drugs or compounds that modulate the TSRX protein activity or expression as well as to treat disorders characterized by insufficient or excessive production of TSRX protein or production of TSRX protein forms that have decreased or aberrant activity compared to TSRX wild-type protein. In addition, the anti-TSRX antibodies of the invention can be used to detect and isolate TSRX proteins and modulate TSRX activity.

Screening Assays

The invention provides a method (also referred to herein as a "screening assay") for identifying modulators, *i.e.*, candidate or test compounds or agents (e.g., peptides, peptidomimetics, small molecules or other drugs) that bind to TSRX proteins or have a stimulatory or inhibitory effect on, *e.g.*, TSRX protein expression or TSRX protein activity. The invention also includes compounds identified in the screening assays described herein.

In one embodiment, the invention provides assays for screening candidate or test compounds which bind to or modulate the activity of the membrane-bound form of a TSRX protein or polypeptide or biologically-active portion thereof. The test compounds of the invention can be obtained using any of the numerous approaches in combinatorial library methods known in the art, including: biological libraries; spatially addressable parallel solid phase or solution phase libraries; synthetic library methods requiring deconvolution; the "one-bead one-compound" library method; and synthetic library methods using affinity chromatography selection. The biological library approach is limited to peptide libraries, while the other four approaches are applicable to peptide, non-peptide oligomer or small molecule libraries of compounds. *See, e.g.*, Lam, 1997. *Anticancer Drug Design* 12: 145.

A "small molecule" as used herein, is meant to refer to a composition that has a molecular weight of less than about 5 kD and most preferably less than about 4 kD. Small molecules can be, *e.g.*, nucleic acids, peptides, polypeptides, peptidomimetics, carbohydrates, lipids or other organic or inorganic molecules. Libraries of chemical and/or biological mixtures, such as fungal, bacterial, or algal extracts, are known in the art and can be screened with any of the assays of the invention.

Examples of methods for the synthesis of molecular libraries can be found in the art, for example in: DeWitt, *et al.*, 1993. *Proc. Natl. Acad. Sci. U.S.A.* 90: 6909; Erb, *et al.*, 1994. *Proc. Natl. Acad. Sci. USA* 91: 11422; Zuckermann, *et al.*, 1994. *J. Med. Chem.* 37: 2678; Cho, *et al.*, 1993. *Science* 261: 1303; Carrell, *et al.*, 1994. *Angew. Chem. Int. Ed. Engl.* 33: 2059; Carrell, *et al.*, 1994. *Angew. Chem. Int. Ed. Engl.* 33: 2061; and Gallop, *et al.*, 1994. *J. Med. Chem.* 37: 1233.

U.S. Patent 5,233,409), plasmids (Cull, *et al.*, 1992 *Proc. Natl. Acad. Sci. USA* 89: 1865-1869) or on phage (Scott and Smith, 1990, *Science* 249: 386-390; Devlin, 1990 *Science* 249: 404-406; Cwirla, *et al.*, 1990, *Proc. Natl. Acad. Sci. U.S.A.* 87: 6378-6382; Felici, 1991 *J. Mol. Biol.* 222: 301-310; Ladner, U.S. Patent No. 5,233,409.)

In one embodiment, an assay is a cell-based assay in which a cell which expresses a membrane-bound form of TSRX protein, or a biologically-active portion thereof, on the cell surface is contacted with a test compound and the ability of the test compound to bind to a TSRX protein determined. The cell, for example, can of mammalian origin or a yeast cell. Determining the ability of the test compound to bind to the TSRX protein can be accomplished, for example, by coupling the test compound with a radioisotope or enzymatic label such that binding of the test compound to the TSRX protein or biologically-active portion thereof can be determined by detecting the labeled compound in a complex. For example, test compounds can be labeled with ^{125}I, ^{35}S, ^{14}C, or ^{3}H, either directly or indirectly, and the radioisotope detected by direct counting of radioemission or by scintillation counting. Alternatively, test compounds can be enzymatically-labeled with, for example, horseradish peroxidase, alkaline phosphatase, or luciferase, and the enzymatic label detected by determination of conversion of an appropriate substrate to product. In one embodiment, the assay comprises contacting a cell which expresses a membrane-bound form of TSRX protein, or a biologically-active portion thereof, on the cell surface with a known compound which binds TSRX to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with a TSRX protein, wherein determining the ability of the test compound to interact with a TSRX protein comprises determining the ability of the test compound to preferentially bind to TSRX protein or a biologically-active portion thereof as compared to the known compound.

In another embodiment, an assay is a cell-based assay comprising contacting a cell expressing a membrane-bound form of TSRX protein, or a biologically-active portion thereof, on the cell surface with a test compound and determining the ability of the test compound to modulate (e.g. stimulate or inhibit) the activity of the TSRX protein or biologically-active portion thereof. Determining the ability of the test compound to modulate the activity of

molecule". As used herein, a "target molecule" is a molecule with which a TSRX protein binds or interacts in nature, for example, a molecule on the surface of a cell which expresses a TSRX interacting protein, a molecule on the surface of a second cell, a molecule in the extracellular milieu, a molecule associated with the internal surface of a cell membrane or a cytoplasmic molecule. A TSRX target molecule can be a non-TSRX molecule or a TSRX protein or polypeptide of the invention. In one embodiment, a TSRX target molecule is a component of a signal transduction pathway that facilitates transduction of an extracellular signal (e.g. a signal generated by binding of a compound to a membrane-bound TSRX molecule) through the cell membrane and into the cell. The target, for example, can be a second intercellular protein that has catalytic activity or a protein that facilitates the association of downstream signaling molecules with TSRX.

Determining the ability of the TSRX protein to bind to or interact with a TSRX target molecule can be accomplished by one of the methods described above for determining direct binding. In one embodiment, determining the ability of the TSRX protein to bind to or interact with a TSRX target molecule can be accomplished by determining the activity of the target molecule. For example, the activity of the target molecule can be determined by detecting induction of a cellular second messenger of the target (*i.e.* intracellular Ca^{2+}, diacylglycerol, IP_3, etc.), detecting catalytic enzymatic activity of the target an appropriate substrate, detecting the induction of a reporter gene (comprising a TSRX-responsive regulatory element operatively linked to a nucleic acid encoding a detectable marker, e.g., luciferase), or detecting a cellular response, for example, cell survival, cellular differentiation, or cell proliferation.

In yet another embodiment, an assay of the invention is a cell-free assay comprising contacting a TSRX protein or biologically-active portion thereof with a test compound and determining the ability of the test compound to bind to the TSRX protein or biologically-active portion thereof. Binding of the test compound to the TSRX protein can be determined either directly or indirectly as described above. In one such embodiment, the assay comprises contacting the TSRX protein or biologically-active portion thereof with a known compound which binds TSRX to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with a TSRX protein.

comprises determining the ability of the test compound to preferentially bind to TSRX or biologically-active portion thereof as compared to the known compound.

In still another embodiment, an assay is a cell-free assay comprising contacting TSRX protein or biologically-active portion thereof with a test compound and determining the ability of the test compound to modulate (e.g. stimulate or inhibit) the activity of the TSRX protein or biologically-active portion thereof. Determining the ability of the test compound to modulate the activity of TSRX can be accomplished, for example, by determining the ability of the TSRX protein to bind to a TSRX target molecule by one of the methods described above for determining direct binding. In an alternative embodiment, determining the ability of the test compound to modulate the activity of TSRX protein can be accomplished by determining the ability of the TSRX protein further modulate a TSRX target molecule. For example, the catalytic enzymatic activity of the target molecule on an appropriate substrate can be determined as described above.

In yet another embodiment, the cell-free assay comprises contacting the TSRX protein or biologically-active portion thereof with a known compound which binds TSRX protein to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with a TSRX protein, wherein determining the ability of the test compound to interact with a TSRX protein comprises determining the ability of the TSRX protein to preferentially bind to or modulate the activity of a TSRX target molecule.

The cell-free assays of the invention are amenable to use of both the soluble form or the membrane-bound form of TSRX protein. In the case of cell-free assays comprising the membrane-bound form of TSRX protein, it may be desirable to utilize a solubilizing agent such that the membrane-bound form of TSRX protein is maintained in solution. Examples of such solubilizing agents include non-ionic detergents such as n-octylglucoside, n-dodecylglucoside, n-dodecyl maltoside, octanoyl-N-methylglucamide, decanoyl-N-methylglucamide, Triton® X-100, Triton® X-114, Thesit®, Isotridecypolyethylene glycol ether$_n$, N-dodecyl--N,N-dimethyl-3-ammonio-1-propane sulfonate, 3-[3-cholamidopropyl) dimethylamminiol-1-propane sulfonate (CHAPS), or

In more than one embodiment of the above assay methods of the invention, it may be desirable to immobilize either TSRX protein or its target molecule to facilitate separation of complexed from uncomplexed forms of one or both of the proteins, as well as to accommodate automation of the assay. Binding of a test compound to TSRX protein, or interaction of TSRX protein with a target molecule in the presence and absence of a candidate compound, can be accomplished in any vessel suitable for containing the reactants. Examples of such vessels include microtiter plates, test tubes, and micro-centrifuge tubes. In one embodiment, a fusion protein can be provided that adds a domain that allows one or both of the proteins to be bound to a matrix. For example, GST-TSRX fusion proteins or GST-target fusion proteins can be adsorbed onto glutathione sepharose beads (Sigma Chemical, St. Louis, MO) or glutathione derivatized microtiter plates, that are then combined with the test compound or the test compound and either the non-adsorbed target protein or TSRX protein, and the mixture is incubated under conditions conducive to complex formation (e.g., at physiological conditions for salt and pH). Following incubation, the beads or microtiter plate wells are washed to remove any unbound components, the matrix immobilized in the case of beads, complex determined either directly or indirectly, for example, as described, *supra*. Alternatively, the complexes can be dissociated from the matrix, and the level of TSRX protein binding or activity determined using standard techniques.

Other techniques for immobilizing proteins on matrices can also be used in the screening assays of the invention. For example, either the TSRX protein or its target molecule can be immobilized utilizing conjugation of biotin and streptavidin. Biotinylated TSRX protein or target molecules can be prepared from biotin-NHS (N-hydroxy-succinimide) using techniques well-known within the art (e.g., biotinylation kit, Pierce Chemicals, Rockford, Ill.), and immobilized in the wells of streptavidin-coated 96 well plates (Pierce Chemical). Alternatively, antibodies reactive with TSRX protein or target molecules, but which do not interfere with binding of the TSRX protein to its target molecule, can be derivatized to the wells of the plate, and unbound target or TSRX protein trapped in the wells by antibody conjugation. Methods for detecting such complexes, in addition to those described above for the GST-immobilized complexes, include immunodetection of complexes using antibodies reactive with the TSRX protein or target molecule, as well as enzyme-linked assays that rely

In another embodiment, modulators of TSRX protein expression are identified in a method wherein a cell is contacted with a candidate compound and the expression of TSRX mRNA or protein in the cell is determined. The level of expression of TSRX mRNA or protein in the presence of the candidate compound is compared to the level of expression of TSRX mRNA or protein in the absence of the candidate compound. The candidate compound can then be identified as a modulator of TSRX mRNA or protein expression based upon this comparison. For example, when expression of TSRX mRNA or protein is greater (*i.e.*, statistically significantly greater) in the presence of the candidate compound than in its absence, the candidate compound is identified as a stimulator of TSRX mRNA or protein expression. Alternatively, when expression of TSRX mRNA or protein is less (statistically significantly less) in the presence of the candidate compound than in its absence, the candidate compound is identified as an inhibitor of TSRX mRNA or protein expression. The level of TSRX mRNA or protein expression in the cells can be determined by methods described herein for detecting TSRX mRNA or protein.

In yet another aspect of the invention, the TSRX proteins can be used as "bait proteins" in a two-hybrid assay or three hybrid assay (*see, e.g.*, U.S. Patent No. 5,283,317; Zervos, *et al.*, 1993, *Cell* 72: 223-232; Madura, *et al.*, 1993, *J. Biol. Chem.* 268: 12046-12054; Bartel, *et al.*, 1993, *Biotechniques* 14: 920-924; Iwabuchi, *et al.*, 1993, *Oncogene* 8: 1693-1696; and Brent WO 94/10300), to identify other proteins that bind to or interact with TSRX ("TSRX-binding proteins" or "TSRX-bp") and modulate TSRX activity. Such TSRX-binding proteins are also likely to be involved in the propagation of signals by the TSRX proteins as, for example, upstream or downstream elements of the TSRX pathway.

The two-hybrid system is based on the modular nature of most transcription factors, which consist of separable DNA-binding and activation domains. Briefly, the assay utilizes two different DNA constructs. In one construct, the gene that codes for TSRX is fused to a gene encoding the DNA binding domain of a known transcription factor (*e.g.*, GAL-4). In the other construct, a DNA sequence, from a library of DNA sequences, that encodes an unidentified protein ("prey" or "sample") is fused to a gene that codes for the activation domain of the known transcription factor. If the "bait" and the "prey" proteins are able to

transcription of a reporter gene (e.g., LacZ) that is operably linked to a transcriptional regulatory site responsive to the transcription factor. Expression of the reporter gene can be detected and cell colonies containing the functional transcription factor can be isolated and used to obtain the cloned gene that encodes the protein which interacts with TSRX.

The invention further pertains to novel agents identified by the aforementioned screening assays and uses thereof for treatments as described herein.

Detection Assays

Portions or fragments of the cDNA sequences identified herein (and the corresponding complete gene sequences) can be used in numerous ways as polynucleotide reagents. By way of example, and not of limitation, these sequences can be used to: (i) identify an individual from a minute biological sample (tissue typing); and (ii) aid in forensic identification of a biological sample. Some of these applications are described in the subsections, below.

Tissue Typing

The TSRX sequences of the invention can be used to identify individuals from minute biological samples. In this technique, an individual's genomic DNA is digested with one or more restriction enzymes, and probed on a Southern blot to yield unique bands for identification. The sequences of the invention are useful as additional DNA markers for RFLP ("restriction fragment length polymorphisms," described in U.S. Patent No. 5,272,057).

Furthermore, the sequences of the invention can be used to provide an alternative technique that determines the actual base-by-base DNA sequence of selected portions of an individual's genome. Thus, the TSRX sequences described herein can be used to prepare two PCR primers from the 5'- and 3'-termini of the sequences. These primers can then be used to amplify an individual's DNA and subsequently sequence it.

Panels of corresponding DNA sequences from individuals, prepared in this manner, can provide unique individual identifications, as each individual will have a unique set of such DNA sequences due to allelic differences. The sequences of the invention can be used to obtain such identification sequences from individuals and from tissue. The TSRX sequences

regions. It is estimated that allelic variation between individual humans occurs with a frequency of about once per each 500 bases. Much of the allelic variation is due to single nucleotide polymorphisms (SNPs), which include restriction fragment length polymorphisms (RFLPs).

Each of the sequences described herein can, to some degree, be used as a standard against which DNA from an individual can be compared for identification purposes. Because greater numbers of polymorphisms occur in the noncoding regions, fewer sequences are necessary to differentiate individuals. The noncoding sequences can comfortably provide positive individual identification with a panel of perhaps 10 to 1,000 primers that each yield a noncoding amplified sequence of 100 bases. If predicted coding sequences, such as those in SEQ ID NO: 1 and 3 are used, a more appropriate number of primers for positive individual identification would be 500-2,000.

Predictive Medicine

The invention also pertains to the field of predictive medicine in which diagnostic assays, prognostic assays, pharmacogenomics, and monitoring clinical trials are used for prognostic (predictive) purposes to thereby treat an individual prophylactically. Accordingly, one aspect of the invention relates to diagnostic assays for determining TSRX protein and/or nucleic acid expression as well as TSRX activity, in the context of a biological sample (e.g., blood, serum, cells, tissue) to thereby determine whether an individual is afflicted with a disease or disorder, or is at risk of developing a disorder, associated with aberrant TSRX expression or activity. Disorders associated with aberrant TSRX expression of activity include for example, cancer, rheumatoid arthritis and ocular neovasularisation. The invention also provides for prognostic (or predictive) assays for determining whether an individual is at risk of developing a disorder associated with TSRX protein, nucleic acid expression or activity. For example, mutations in a TSRX gene can be assayed in a biological sample. Such assays can be used for prognostic or predictive purpose to thereby prophylactically treat an individual prior to the onset of a disorder characterized by or associated with TSRX protein, nucleic acid expression, or biological activity.

prophylactic agents for that individual (referred to herein as "pharmacogenomics") Pharmacogenomics allows for the selection of agents (e.g., drugs) for therapeutic or prophylactic treatment of an individual based on the genotype of the individual (e.g., the genotype of the individual examined to determine the ability of the individual to respond to a particular agent.)

Yet another aspect of the invention pertains to monitoring the influence of agents (e.g., drugs, compounds) on the expression or activity of TSRX in clinical trials

These and other agents are described in further detail in the following sections.

Diagnostic Assays

An exemplary method for detecting the presence or absence of TSRX in a biological sample involves obtaining a biological sample from a test subject and contacting the biological sample with a compound or an agent capable of detecting TSRX protein or nucleic acid (e.g., mRNA, genomic DNA) that encodes TSRX protein such that the presence of TSRX is detected in the biological sample. An agent for detecting TSRX mRNA or genomic DNA is a labeled nucleic acid probe capable of hybridizing to TSRX mRNA or genomic DNA. The nucleic acid probe can be, for example, a full-length TSRX nucleic acid, such as the nucleic acid of SEQ ID NO: 1 and 3, or a portion thereof, such as an oligonucleotide of at least 15, 30, 50, 100, 250 or 500 nucleotides in length and sufficient to specifically hybridize under stringent conditions to TSRX mRNA or genomic DNA. Other suitable probes for use in the diagnostic assays of the invention are described herein.

One agent for detecting TSRX protein is an antibody capable of binding to TSRX protein, preferably an antibody with a detectable label. Antibodies directed against a protein of the invention may be used in methods known within the art relating to the localization and/or quantitation of the protein (e.g., for use in measuring levels of the protein within appropriate physiological samples, for use in diagnostic methods, for use in imaging the protein, and the like). In a given embodiment, antibodies against the proteins, or derivatives, fragments, analogs or homologs thereof, that contain the antigen binding domain, are utilized as pharmacologically-active compounds.

an antibody can facilitate the purification of the natural protein antigen from cells and of recombinantly produced antigen expressed in host cells. Moreover, such an antibody can be used to detect the antigenic protein (e.g., in a cellular lysate or cell supernatant) in order to evaluate the abundance and pattern of expression of the antigenic protein. Antibodies directed against the protein can be used diagnostically to monitor protein levels in tissue as part of a clinical testing procedure, e.g., to, for example, determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling (i.e., physically linking) the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, -galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin, and examples of suitable radioactive material include ^{125}I, ^{131}I, ^{35}S or ^{3}H.

Antibodies can be polyclonal, or more preferably, monoclonal. An intact antibody, or a fragment thereof (e.g., Fab or F(ab')$_2$) can be used. The term "labeled", with regard to the probe or antibody, is intended to encompass direct labeling of the probe or antibody by coupling (i.e., physically linking) a detectable substance to the probe or antibody, as well as indirect labeling of the probe or antibody by reactivity with another reagent that is directly labeled. Examples of indirect labeling include detection of a primary antibody using a fluorescently-labeled secondary antibody and end-labeling of a DNA probe with biotin such that it can be detected with fluorescently-labeled streptavidin. The term "biological sample" is intended to include tissues, cells and biological fluids isolated from a subject, as well as tissues, cells and fluids present within a subject. That is, the detection method of the invention can be used to detect TSRX mRNA, protein, or genomic DNA in a biological sample *in vitro* as well as *in vivo*. For example, *in vitro* techniques for detection of TSRX mRNA include Northern hybridizations and *in situ* hybridizations. *In vitro* techniques for detection of TSRX

genomic DNA include Southern hybridizations. Furthermore, *in vivo* techniques for detection of TSRX protein include introducing into a subject a labeled anti-TSRX antibody. For example, the antibody can be labeled with a radioactive marker whose presence and location in a subject can be detected by standard imaging techniques.

In one embodiment, the biological sample contains protein molecules from the test subject. Alternatively, the biological sample can contain mRNA molecules from the test subject or genomic DNA molecules from the test subject. A preferred biological sample is a peripheral blood leukocyte sample isolated by conventional means from a subject.

In one embodiment, the methods further involve obtaining a control biological sample from a control subject, contacting the control sample with a compound or agent capable of detecting TSRX protein, mRNA, or genomic DNA, such that the presence of TSRX protein, mRNA or genomic DNA is detected in the biological sample, and comparing the presence of TSRX protein, mRNA or genomic DNA in the control sample with the presence of TSRX protein, mRNA or genomic DNA in the test sample.

The invention also encompasses kits for detecting the presence of TSRX in a biological sample. For example, the kit can comprise: a labeled compound or agent capable of detecting TSRX protein or mRNA in a biological sample; means for determining the amount of TSRX in the sample; and means for comparing the amount of TSRX in the sample with a standard. The compound or agent can be packaged in a suitable container. The kit can further comprise instructions for using the kit to detect TSRX protein or nucleic acid.

Prognostic Assays

The diagnostic methods described herein can furthermore be utilized to identify subjects having or at risk of developing a disease or disorder associated with aberrant TSRX expression or activity. For example, the assays described herein, such as the preceding diagnostic assays or the following assays, can be utilized to identify a subject having or at risk of developing a disorder associated with TSRX protein, nucleic acid expression or activity. Such disorders include for example, cancer, rheumatoid arthritis and ocular neovascularisation. Alternatively, the prognostic assays can be utilized to identify a subject having or at risk for

sample is obtained from a subject and TSRX protein or nucleic acid (e.g., mRNA, genomic DNA) is detected, wherein the presence of TSRX protein or nucleic acid is diagnostic for a subject having or at risk of developing a disease or disorder associated with aberrant TSRX expression or activity. As used herein, a "test sample" refers to a biological sample obtained from a subject of interest. For example, a test sample can be a biological fluid (e.g., serum), cell sample, or tissue.

Furthermore, the prognostic assays described herein can be used to determine whether a subject can be administered an agent (e.g., an agonist, antagonist, peptidomimetic, protein, peptide, nucleic acid, small molecule, or other drug candidate) to treat a disease or disorder associated with aberrant TSRX expression or activity. For example, such methods can be used to determine whether a subject can be effectively treated with an agent for a disorder. Thus, the invention provides methods for determining whether a subject can be effectively treated with an agent for a disorder associated with aberrant TSRX expression or activity in which a test sample is obtained and TSRX protein or nucleic acid is detected (e.g., wherein the presence of TSRX protein or nucleic acid is diagnostic for a subject that can be administered the agent to treat a disorder associated with aberrant TSRX expression or activity).

The methods of the invention can also be used to detect genetic lesions in a TSRX gene, thereby determining if a subject with the lesioned gene is at risk for a disorder characterized by aberrant cell proliferation and/or differentiation. In various embodiments, the methods include detecting, in a sample of cells from the subject, the presence or absence of a genetic lesion characterized by at least one of an alteration affecting the integrity of a gene encoding a TSRX-protein, or the misexpression of the TSRX gene. For example, such genetic lesions can be detected by ascertaining the existence of at least one of: (i) a deletion of one or more nucleotides from a TSRX gene; (ii) an addition of one or more nucleotides to a TSRX gene; (iii) a substitution of one or more nucleotides of a TSRX gene; (iv) a chromosomal rearrangement of a TSRX gene; (v) an alteration in the level of a messenger RNA transcript of a TSRX gene; (vi) aberrant modification of a TSRX gene, such as of the methylation pattern of the genomic DNA; (vii) the presence of a non-wild-type splicing pattern of a messenger RNA transcript of a TSRX gene; (viii) a non-wild-type level of a TSRX protein; (ix) allelic loss of a

used for detecting lesions in a TSRX gene. A preferred biological sample is a peripheral blood leukocyte sample isolated by conventional means from a subject. However, any biological sample containing nucleated cells may be used, including, for example, buccal mucosal cells.

In certain embodiments, detection of the lesion involves the use of a probe primer in a polymerase chain reaction (PCR) (see, e.g., U.S. Patent Nos. 4,683,195 and 4,683,202), such as anchor PCR or RACE PCR, or, alternatively, in a ligation chain reaction (LCR) (see, e.g., Landegran, *et al.*, 1988, *Science* 241: 1077-1080; and Nakazawa, *et al.*, 1994, *Proc. Natl. Acad. Sci. USA* 91: 360-364), the latter of which can be particularly useful for detecting point mutations in the TSRX-gene (see, Abravaya, *et al.*, 1995, *Nucl. Acids Res.* 23: 675-682). This method can include the steps of collecting a sample of cells from a patient, isolating nucleic acid (e.g., genomic, mRNA or both) from the cells of the sample, contacting the nucleic acid sample with one or more primers that specifically hybridize to a TSRX gene under conditions such that hybridization and amplification of the TSRX gene (if present) occurs, and detecting the presence or absence of an amplification product, or detecting the size of the amplification product and comparing the length to a control sample. It is anticipated that PCR and/or LCR may be desirable to use as a preliminary amplification step in conjunction with any of the techniques used for detecting mutations described herein.

Alternative amplification methods include: self sustained sequence replication (see Guatelli, *et al.*, 1990, *Proc. Natl. Acad. Sci. USA* 87: 1874-1878), transcriptional amplification system (see, Kwoh, *et al.*, 1989, *Proc. Natl. Acad. Sci. USA* 86: 1173-1177); Qβ Replicase (see, Lizardi, *et al.* 1988, *BioTechnology* 6: 1197), or any other nucleic acid amplification method, followed by the detection of the amplified molecules using techniques well known to those of skill in the art. These detection schemes are especially useful for the detection of nucleic acid molecules if such molecules are present in very low numbers.

In an alternative embodiment, mutations in a TSRX gene from a sample cell can be identified by alterations in restriction enzyme cleavage patterns. For example, sample and control DNA is isolated, amplified (optionally), digested with one or more restriction endonucleases, and fragment length sizes are determined by gel electrophoresis and compared. Differences in fragment length sizes between sample and control DNA indicates mutations in

5,493,531) can be used to score for the presence of specific mutations by development or loss of a ribozyme cleavage site.

In other embodiments, genetic mutations in TSRX can be identified by hybridizing a sample and control nucleic acids, *e.g.*, DNA or RNA, to high-density arrays containing hundreds or thousands of oligonucleotides probes. *See, e.g.*, Cronin, *et al.*, 1996, *Human Mutation* 7: 244-255; Kozal, *et al.*, 1996, *Nat. Med.* 2: 753-759. For example, genetic mutations in TSRX can be identified in two dimensional arrays containing light-generated DNA probes as described in Cronin, *et al.*, *supra*. Briefly, a first hybridization array of probes can be used to scan through long stretches of DNA in a sample and control to identify base changes between the sequences by making linear arrays of sequential overlapping probes. This step allows the identification of point mutations. This is followed by a second hybridization array that allows the characterization of specific mutations by using smaller, specialized probe arrays complementary to all variants or mutations detected. Each mutation array is composed of parallel probe sets, one complementary to the wild-type gene and the other complementary to the mutant gene.

In yet another embodiment, any of a variety of sequencing reactions known in the art can be used to directly sequence the TSRX gene and detect mutations by comparing the sequence of the sample TSRX with the corresponding wild-type (control) sequence. Examples of sequencing reactions include those based on techniques developed by Maxim and Gilbert, 1977, *Proc. Natl. Acad. Sci. USA* 74: 560 or Sanger, 1977, *Proc. Natl. Acad. Sci. USA* 74: 5463. It is also contemplated that any of a variety of automated sequencing procedures can be utilized when performing the diagnostic assays (*see, e.g.*, Naeve, *et al.*, 1995, *Biotechniques* 19: 448), including sequencing by mass spectrometry (see, e.g., PCT International Publication No. WO 94/16101; Cohen, *et al.*, 1996, *Adv. Chromatography* 36: 127-162; and Griffin, *et al.*, 1993, *Appl. Biochem. Biotechnol.* 38: 147-159).

Other methods for detecting mutations in the TSRX gene include methods in which protection from cleavage agents is used to detect mismatched bases in RNA/RNA or RNA/DNA heteroduplexes. *See, e.g.*, Myers, *et al.*, 1985, *Science* 230: 1242. In general, the art technique of "mismatch cleavage" starts by providing heteroduplexes of formed by

with an agent that cleaves single-stranded regions of the duplex such as which will exist due to basepair mismatches between the control and sample strands. For instance, RNA DNA duplexes can be treated with RNase and DNA DNA hybrids treated with S1 nuclease to enzymatically digesting the mismatched regions. In other embodiments, either DNA DNA or RNA DNA duplexes can be treated with hydroxylamine or osmium tetroxide and with piperidine in order to digest mismatched regions. After digestion of the mismatched regions, the resulting material is then separated by size on denaturing polyacrylamide gels to determine the site of mutation. *See, e.g.* Cotton, *et al.*, 1988, *Proc. Natl. Acad. Sci. USA* 85: 4397; Saleeba, *et al.*, 1992, *Methods Enzymol.* 217: 286-295. In an embodiment, the control DNA or RNA can be labeled for detection.

In still another embodiment, the mismatch cleavage reaction employs one or more proteins that recognize mismatched base pairs in double-stranded DNA (so called "DNA mismatch repair" enzymes) in defined systems for detecting and mapping point mutations in TSRX cDNAs obtained from samples of cells. For example, the mutY enzyme of *E. coli* cleaves A at G A mismatches and the thymidine DNA glycosylase from HeLa cells cleaves T at G T mismatches. *See, e.g.*, Hsu, *et al.*, 1994, *Carcinogenesis* 15: 1657-1662. According to an exemplary embodiment, a probe based on a TSRX sequence, e.g., a wild-type TSRX sequence, is hybridized to a cDNA or other DNA product from a test cell(s). The duplex is treated with a DNA mismatch repair enzyme, and the cleavage products, if any, can be detected from electrophoresis protocols or the like. *See, e.g.*, U.S. Patent No. 5,459,039.

In other embodiments, alterations in electrophoretic mobility will be used to identify mutations in TSRX genes. For example, single strand conformation polymorphism (SSCP) may be used to detect differences in electrophoretic mobility between mutant and wild type nucleic acids. *See, e.g.*, Orita, *et al.*, 1989, *Proc. Natl. Acad. Sci. USA* 86: 2766; Cotton, 1993, *Mutat. Res.* 285: 125-144; Hayashi, 1992, *Genet. Anal. Tech. Appl.* 9: 73-79. Single-stranded DNA fragments of sample and control TSRX nucleic acids will be denatured and allowed to renature. The secondary structure of single-stranded nucleic acids varies according to sequence, the resulting alteration in electrophoretic mobility enables the detection of even a single base change. The DNA fragments may be labeled or detected with labeled

the subject method utilizes heteroduplex analysis to separate double stranded heteroduplex molecules on the basis of changes in electrophoretic mobility. *See, e.g.*, Keen, *et al.*, 1991 *Trends Genet.* 7: 5.

In yet another embodiment, the movement of mutant or wild-type fragments in polyacrylamide gels containing a gradient of denaturant is assayed using denaturing gradient gel electrophoresis (DGGE). *See, e.g.*, Myers, *et al.*, 1985. *Nature* 313: 495. When DGGE is used as the method of analysis, DNA will be modified to insure that it does not completely denature, for example by adding a GC clamp of approximately 40 bp of high-melting GC-rich DNA by PCR. In a further embodiment, a temperature gradient is used in place of a denaturing gradient to identify differences in the mobility of control and sample DNA. *See e.g.*, Rosenbaum and Reissner, 1987, *Biophys. Chem.* 265: 12753.

Examples of other techniques for detecting point mutations include, but are not limited to, selective oligonucleotide hybridization, selective amplification, or selective primer extension. For example, oligonucleotide primers may be prepared in which the known mutation is placed centrally and then hybridized to target DNA under conditions that permit hybridization only if a perfect match is found. *See, e.g.*, Saiki, *et al.*, 1986. *Nature* 324: 163; Saiki, *et al.*, 1989. *Proc. Natl. Acad. Sci. USA* 86: 6230. Such allele specific oligonucleotides are hybridized to PCR amplified target DNA or a number of different mutations when the oligonucleotides are attached to the hybridizing membrane and hybridized with labeled target DNA.

Alternatively, allele specific amplification technology that depends on selective PCR amplification may be used in conjunction with the instant invention. Oligonucleotides used as primers for specific amplification may carry the mutation of interest in the center of the molecule (so that amplification depends on differential hybridization; *see, e.g.*, Gibbs, *et al.*, 1989. *Nucl. Acids Res.* 17: 2437-2448) or at the extreme 3'-terminus of one primer where, under appropriate conditions, mismatch can prevent, or reduce polymerase extension (*see, e.g.*, Prossner, 1993. *Tibtech.* 11: 238). In addition it may be desirable to introduce a novel restriction site in the region of the mutation to create cleavage-based detection. *See, e.g.*, Gasparini, *et al.*, 1992. *Mol. Cell Probes* 6: 1. It is anticipated that in certain embodiments

perfect match at the 3'-terminus of the 5' sequence, making it possible to detect the presence of a known mutation at a specific site by looking for the presence or absence of amplification.

The methods described herein may be performed, for example, by utilizing pre-packaged diagnostic kits comprising at least one probe nucleic acid or antibody reagent described herein, which may be conveniently used, e.g., in clinical settings to diagnose patients exhibiting symptoms or family history of a disease or illness involving a TSRX gene.

Furthermore, any cell type or tissue, preferably peripheral blood leukocytes, in which TSRX is expressed may be utilized in the prognostic assays described herein. However, any biological sample containing nucleated cells may be used, including, for example, buccal mucosal cells.

Pharmacogenomics

Agents, or modulators that have a stimulatory or inhibitory effect on TSRX activity (e.g., TSRX gene expression), as identified by a screening assay described herein can be administered to individuals to treat (prophylactically or therapeutically) disorders (e.g., cancer, rheumatoid arthritis and ocular neovasularisation). In conjunction with such treatment, the pharmacogenomics (*i.e.*, the study of the relationship between an individual's genotype and that individual's response to a foreign compound or drug) of the individual may be considered. Differences in metabolism of therapeutics can lead to severe toxicity or therapeutic failure by altering the relation between dose and blood concentration of the pharmacologically active drug. Thus, the pharmacogenomics of the individual permits the selection of effective agents (e.g., drugs) for prophylactic or therapeutic treatments based on a consideration of the individual's genotype. Such pharmacogenomics can further be used to determine appropriate dosages and therapeutic regimens. Accordingly, the activity of TSRX protein, expression of TSRX nucleic acid, or mutation content of TSRX genes in an individual can be determined to thereby select appropriate agent(s) for therapeutic or prophylactic treatment of the individual.

Pharmacogenomics deals with clinically significant hereditary variations in the response to drugs due to altered drug disposition and abnormal action in affected persons. See

differentiated. Genetic conditions transmitted as a single factor altering the way drugs act on the body (altered drug action) or genetic conditions transmitted as single factors altering the way the body acts on drugs (altered drug metabolism). These pharmacogenetic conditions can occur either as rare defects or as polymorphisms. For example, glucose-6-phosphate dehydrogenase (G6PD) deficiency is a common inherited enzymopathy in which the main clinical complication is hemolysis after ingestion of oxidant drugs (anti-malarials, sulfonamides, analgesics, nitrofurans) and consumption of fava beans.

As an illustrative embodiment, the activity of drug metabolizing enzymes is a major determinant of both the intensity and duration of drug action. The discovery of genetic polymorphisms of drug metabolizing enzymes (e.g., N-acetyltransferase 2 (NAT 2) and cytochrome P450 enzymes CYP2D6 and CYP2C19) has provided an explanation as to why some patients do not obtain the expected drug effects or show exaggerated drug response and serious toxicity after taking the standard and safe dose of a drug. These polymorphisms are expressed in two phenotypes in the population, the extensive metabolizer (EM) and poor metabolizer (PM). The prevalence of PM is different among different populations. For example, the gene coding for CYP2D6 is highly polymorphic and several mutations have been identified in PM, which all lead to the absence of functional CYP2D6. Poor metabolizers of CYP2D6 and CYP2C19 quite frequently experience exaggerated drug response and side effects when they receive standard doses. If a metabolite is the active therapeutic moiety, PM show no therapeutic response, as demonstrated for the analgesic effect of codeine mediated by its CYP2D6-formed metabolite morphine. At the other extreme are the so called ultra-rapid metabolizers who do not respond to standard doses. Recently, the molecular basis of ultra-rapid metabolism has been identified to be due to CYP2D6 gene amplification.

Thus, the activity of TSRX protein, expression of TSRX nucleic acid, or mutation content of TSRX genes in an individual can be determined to thereby select appropriate agent(s) for therapeutic or prophylactic treatment of the individual. In addition, pharmacogenetic studies can be used to apply genotyping of polymorphic alleles encoding drug-metabolizing enzymes to the identification of an individual's drug responsiveness phenotype. This knowledge, when applied to dosing or drug selection, can avoid adverse

treating a subject with a TSRX modulator, such as a modulator identified by one of the exemplary screening assays described herein

Monitoring of Effects During Clinical Trials

Monitoring the influence of agents (*e.g.*, drugs, compounds) on the expression or activity of TSRX (*e.g.*, the ability to modulate aberrant cell proliferation) can be applied not only in basic drug screening, but also in clinical trials. For example, the effectiveness of an agent determined by a screening assay as described herein to increase TSRX gene expression, protein levels, or upregulate TSRX activity, can be monitored in clinical trails of subjects exhibiting decreased TSRX gene expression, protein levels, or downregulated TSRX activity. Alternatively, the effectiveness of an agent determined by a screening assay to decrease TSRX gene expression, protein levels, or downregulate TSRX activity, can be monitored in clinical trails of subjects exhibiting increased TSRX gene expression, protein levels, or upregulated TSRX activity. In such clinical trials, the expression or activity of TSRX and, preferably, other genes that have been implicated in, for example, a cellular proliferation or immune disorder can be used as a "read out" or markers of the immune responsiveness of a particular cell

By way of example, and not of limitation, genes, including TSRX, that are modulated in cells by treatment with an agent (*e.g.*, compound, drug or small molecule) that modulates TSRX activity (*e.g.*, identified in a screening assay as described herein) can be identified. Thus, to study the effect of agents on cellular proliferation disorders, for example, in a clinical trial, cells can be isolated and RNA prepared and analyzed for the levels of expression of TSRX and other genes implicated in the disorder. The levels of gene expression (*i.e.*, a gene expression pattern) can be quantified by Northern blot analysis or RT-PCR, as described herein, or alternatively by measuring the amount of protein produced, by one of the methods as described herein, or by measuring the levels of activity of TSRX or other genes. In this manner, the gene expression pattern can serve as a marker, indicative of the physiological response of the cells to the agent. Accordingly, this response state may be determined before, and at various points during, treatment of the individual with the agent.

screening assays described herein) comprising the steps of (*i*) obtaining a pre-administration sample from a subject prior to administration of the agent; (*ii*) detecting the level of expression of a TSRX protein, mRNA, or genomic DNA in the preadministration sample; (*iii*) obtaining one or more post-administration samples from the subject; (*iv*) detecting the level of expression or activity of the TSRX protein, mRNA, or genomic DNA in the post-administration samples; (*v*) comparing the level of expression or activity of the TSRX protein, mRNA, or genomic DNA in the pre-administration sample with the TSRX protein, mRNA, or genomic DNA in the post administration sample or samples; and (*vi*) altering the administration of the agent to the subject accordingly. For example, increased administration of the agent may be desirable to increase the expression or activity of TSRX to higher levels than detected, *i.e.*, to increase the effectiveness of the agent. Alternatively, decreased administration of the agent may be desirable to decrease expression or activity of TSRX to lower levels than detected, *i.e.*, to decrease the effectiveness of the agent.

Methods of Treatment

The invention provides for both prophylactic and therapeutic methods of treating a subject at risk of (or susceptible to) a disorder or having a disorder associated with aberrant TSRX expression or activity. Disorders associated with aberrant TSRX expression of activity include for example, cancer, rheumatoid arthritis and ocular neovasularisation. These methods of treatment will be discussed more fully, below.

Disease and Disorders

Diseases and disorders that are characterized by increased (relative to a subject not suffering from the disease or disorder) levels or biological activity may be treated with Therapeutics that antagonize (*i.e.*, reduce or inhibit) activity. Therapeutics that antagonize activity may be administered in a therapeutic or prophylactic manner. Therapeutics that may be utilized include, but are not limited to: (*i*) an aforementioned peptide, or analogs, derivatives, fragments or homologs thereof; (*ii*) antibodies to an aforementioned peptide; (*iii*) nucleic acids encoding an aforementioned peptide; (*iv*) administration of antisense nucleic acid and nucleic acids that are "dysfunctional" (*i.e.*, due to a heterologous insertion within the

recombination (*see, e.g.*, Capecchi, 1989, *Science* 244: 1288-1292); or (v) modulators (*i.e.*, inhibitors, agonists and antagonists, including additional peptide mimetic of the invention or antibodies specific to a peptide of the invention) that alter the interaction between an aforementioned peptide and its binding partner.

Diseases and disorders that are characterized by decreased (relative to a subject not suffering from the disease or disorder) levels or biological activity may be treated with Therapeutics that increase (*i.e.*, are agonists to) activity. Therapeutics that upregulate activity may be administered in a therapeutic or prophylactic manner. Therapeutics that may be utilized include, but are not limited to, an aforementioned peptide, or analogs, derivatives, fragments or homologs thereof; or an agonist that increases bioavailability.

Increased or decreased levels can be readily detected by quantifying peptide and/or RNA, by obtaining a patient tissue sample (*e.g.*, from biopsy tissue) and assaying it *in vitro* for RNA or peptide levels, structure and/or activity of the expressed peptides (or mRNAs of an aforementioned peptide). Methods that are well-known within the art include, but are not limited to, immunoassays (*e.g.*, by Western blot analysis, immunoprecipitation followed by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis, immunocytochemistry, etc.) and/or hybridization assays to detect expression of mRNAs (*e.g.*, Northern assays, dot blots, *in situ* hybridization, and the like).

Prophylactic Methods

In one aspect, the invention provides a method for preventing, in a subject, a disease or condition associated with an aberrant TSRX expression or activity, by administering to the subject an agent that modulates TSRX expression or at least one TSRX activity. Subjects at risk for a disease that is caused or contributed to by aberrant TSRX expression or activity can be identified by, for example, any or a combination of diagnostic or prognostic assays as described herein. Administration of a prophylactic agent can occur prior to the manifestation of symptoms characteristic of the TSRX aberrancy, such that a disease or disorder is prevented or, alternatively, delayed in its progression. Depending upon the type of TSRX aberrancy, for example, a TSRX agonist or TSRX antagonist agent can be used for treating the subject. The

Therapeutic Methods

Another aspect of the invention pertains to methods of modulating TSRX expression or activity for therapeutic purposes. The modulatory method of the invention involves contacting a cell with an agent that modulates one or more of the activities of TSRX protein activity associated with the cell. An agent that modulates TSRX protein activity can be an agent as described herein, such as a nucleic acid or a protein, a naturally-occurring cognate ligand of a TSRX protein, a peptide, a TSRX peptidomimetic, or other small molecule. In one embodiment, the agent stimulates one or more TSRX protein activity. Examples of such stimulatory agents include active TSRX protein and a nucleic acid molecule encoding TSRX that has been introduced into the cell. In another embodiment, the agent inhibits one or more TSRX protein activity. Examples of such inhibitory agents include antisense TSRX nucleic acid molecules and anti-TSRX antibodies. These modulatory methods can be performed *in vitro* (e.g., by culturing the cell with the agent) or, alternatively, *in vivo* (e.g., by administering the agent to a subject). As such, the invention provides methods of treating an individual afflicted with a disease or disorder characterized by aberrant expression or activity of a TSRX protein or nucleic acid molecule. In one embodiment, the method involves administering an agent (e.g., an agent identified by a screening assay described herein), or combination of agents that modulates (e.g., up-regulates or down-regulates) TSRX expression or activity. In another embodiment, the method involves administering a TSRX protein or nucleic acid molecule as therapy to compensate for reduced or aberrant TSRX expression or activity.

Stimulation of TSRX activity is desirable in situations in which TSRX is abnormally downregulated and or in which increased TSRX activity is likely to have a beneficial effect. One example of such a situation is where a subject has a disorder characterized by aberrant cell proliferation and or differentiation (e.g., cancer or immune associated disorders). Another example of such a situation is where the subject has a gestational disease (e.g., preclampsia).

Antibodies of the invention, including polyclonal, monoclonal, humanized and fully human antibodies, may used as therapeutic agents. Such agents will generally be employed to treat or prevent a disease or pathology in a subject. An antibody preparation, preferably one having high specificity and high affinity for its target antigen, is administered to the subject

molecule and the target antigen in question. In the first instance, administration of the antibody may abrogate or inhibit the binding of the target with an endogenous ligand to which it naturally binds. In this case, the antibody binds to the target and masks a binding site of the naturally occurring ligand, wherein the ligand serves as an effector molecule. Thus the receptor mediates a signal transduction pathway for which ligand is responsible.

Alternatively, the effect may be one in which the antibody elicits a physiological result by virtue of binding to an effector binding site on the target molecule. In this case the target, a receptor having an endogenous ligand which may be absent or defective in the disease or pathology, binds the antibody as a surrogate effector ligand, initiating a receptor-based signal transduction event by the receptor.

A therapeutically effective amount of an antibody of the invention relates generally to the amount needed to achieve a therapeutic objective. As noted above, this may be a binding interaction between the antibody and its target antigen that, in certain cases, interferes with the functioning of the target, and in other cases, promotes a physiological response. The amount required to be administered will furthermore depend on the binding affinity of the antibody for its specific antigen, and will also depend on the rate at which an administered antibody is depleted from the free volume other subject to which it is administered. Common ranges for therapeutically effective dosing of an antibody or antibody fragment of the invention may be, by way of nonlimiting example, from about 0.1 mg/kg body weight to about 50 mg/kg body weight. Common dosing frequencies may range, for example, from twice daily to once a week.

Determination of the Biological Effect of the Therapeutic

In various embodiments of the invention, suitable *in vitro* or *in vivo* assays are performed to determine the effect of a specific Therapeutic and whether its administration is indicated for treatment of the affected tissue.

In various specific embodiments, *in vitro* assays may be performed with representative cells of the type(s) involved in the patient's disorder, to determine if a given Therapeutic exerts the desired effect upon the cell type(s). Compounds for use in therapy may be tested in

testing, any of the animal model system known in the art may be used prior to administration to human subjects.

The invention will be further described in the following examples, which do not limit the scope of the invention described in the claims.

EXAMPLES

EXAMPLE 1: MOLECULAR CLONING OF A FRAGMENT OF 17897469.0.7

In this example, cloning is described for a fragment of the 17897469.0.7 clone. Oligonucleotide primers were designed to PCR amplify the sequence corresponding to amino acids 13-338 of clone 17897469.0.7 (SEQ ID NO:2). The forward primers include an in-frame BglII restriction site: GGA TCC TCC ATA AAT GGA GCT TAT TGC GAG. (SEQ ID NO:13) The reverse primers was CTC GAG CTT CAG GGC CAA GTG ACT GAG. (SEQ ID NO:14)

PCR reactions were performed using a total of 5ng of a mixture containing equal amounts of cDNA derived from human fetal brain, human testis, human mammary and human skeletal muscle tissues,1 mM of each of the forward and reverse primers, 5 mM of dNTP (Clontech Laboratories, Palo Alto CA) and 1 mL of 50xAdvantage-HF 2 polymerase (Clontech Laboratories, Palo Alto CA) in 50 microliter volume. The following reaction conditions were used:

a) 96°C 3 minutes

b) 96°C 30 seconds denaturation

c) 70°C 30 seconds, primer annealing. This temperature was gradually decreased by 1°C/cycle

d) 72°C 1 minute extension

Repeat steps b-d 11 times

e) 96°C 30 seconds denaturation

f) 60°C 30 seconds annealing

h) 72°C 10 minutes final extension

PCR products having an approximate size of 1 kbp were isolated from agarose gel and ligated to pCR2.1 vector (Invitrogen, Carlsbad, CA). The cloned inserts were sequenced, using vector specific, M13 Forward(-40) and M13 Reverse primers as well as the gene specific primers. The primers include:

17897469 S1: AGC GAG CTG TGG TGT CTG (SEQ ID NO:15)

17897469 S2: CAG ACA CCA CAG CTC GCT (SEQ ID NO:16)

17897469 S3: TCT AGC CGT CAC TGC GAC (SEQ ID NO:17)

17897469 S4: GTC GCA GTG ACG GCT AGA (SEQ ID NO:18)

17897469 S5: TGC CGT CCA GAC ACG GTG (SEQ ID NO:19) and

17897469 S6: CAC CGT GTC TGG ACG GCA (SEQ ID NO:20)

The cloned inserts were sequenced and verified as an open reading frame coding for the predicted amino acid sequence. The cloned sequence was determined to be 100% identical to the predicted sequence.

EXAMPLE 2: MOLECULAR CLONING OF A FRAGMENT OF 17897469.0.7

In this example, cloning is described for a fragment of the 17897469.0.7 clone. Oligonucleotide primers were designed to PCR amplify a DNA fragment coding for residues 13-634 of clone 17897469.0.7. The forward primer includes an in frame BamHI restriction site and the reverse primer contains an in frame XhoI restriction site. The sequences of the PCR primers are the following: forward: GGATCCTCCATAAATGGAGCTTATTGGGAG (SEQ ID NO:21) and reverse:CTCGAGCAGGGCCTCCGTGCACTCGTGCGACGC (SEQ ID NO:22).

PCR reactions were performed using a total of 5ng of a mixture containing equal amounts of cDNA derived from human fetal brain, human testis, human mammary and human skeletal muscle tissues,and 1 mM of each of the forward and reverse primers, 5 mM of dNTP (Clontech Laboratories, Palo Alto CA) and 1 mL of 50xAdvantage-HF 2 polymerase (Clontech Laboratories, Palo Alto CA) in 50 microliter volume. The reaction conditions

PCR products having a size of approximately 1.9 kbp were isolated from agarose gel and ligated to pCR2.1 vector (Invitrogen, Carlsbad, CA). The cloned inserts were sequenced, using vector specific, M13 Forward(-40) and M13 Reverse primers as well as the gene specific primers. These primers include:

17897469 S1: AGC GAG CTG TGG TGT CTG (SEQ ID NO:23)

17897469 S2: CAG ACA CCA CAG CTC GCT (SEQ ID NO:24)

17897469 S3: TCT AGC CGT CAC TGC GAC (SEQ ID NO:25)

17897469 S4: GTC GCA GTG ACG GCT AGA (SEQ ID NO:26)

17897469 S5: TGC CGT CCA GAC ACG GTG (SEQ ID NO:27)

17897469 S6: CAC CGT GTC TGG ACG GCA (SEQ ID NO:28)

17897469 S7: CTC AGT CAC TTG GCC CTG (SEQ ID NO:29)

17897469 S8: CTT CAG GGC CAA GTG ACT (SEQ ID NO:30)

17897469 S9: ATC GCC CGT GAC TCG CTG (SEQ ID NO:31)

17897469 S10: CAG CGA GTC ACG GGC GAT (SEQ ID NO:32)

17897469 S11: CTG GAC GAC AGC GCA TGC (SEQ ID NO:33) and

17897469 S12: GCA TGC GCT GTC GTC CAG (SEQ ID NO:34).

The cloned inserts were sequenced and verified as an open reading frame coding for the predicted amino acid sequence. The cloned sequence was determined to be 100% identical to the predicted sequence.

EXAMPLE 3: PREPARATION OF THE INSECT CELL EXPRESSION VECTOR PMELV5HIS

An expression vector, named pMelV5His was constructed for examining the expression of TSRX nucleic acid sequence.

To construct pMelV5His, the oligonucleotide primers, melittin Foward 5'GATCTCCACCATGAAATTCTTAGTCAACGTTGCCCTTGTTTTTATGGTCGTATAC ATTTCTTACATCTATGCGGATCGATGGGGATCCGAGC 3'(SEQ ID NO: 35) and melittin

AAACAAGGGCAACGTTGACTAAGAATTTCATGGTGGA- 3' (SEQ ID NO: 36) were used. These oligonucleotide primers encode a melittin secretion signal peptide and contain the ATG initiation codon. The oligonucleotides primers were annealed with pBlueBac4.5 (Invitrogen, Carlsbad, CA) that had been previously digested with BamHI and XhoI to produce pBlueBac4.5MelI. A XhoI-PmeI fragment containing V5 and oxHis tags wereisolated from pcDNA3.1(A) (Invitrogen, Carlsbad, CA) and ligated into pBlueBac4.5MelI. The resulting vector was named pMelV5His, and is useful for expression of heterologous proteins in Sf9cells.

EXAMPLE 4: EXPRESSION OF 17897469 IN INSECT CELLS

A BamHI-XhoI fragment containing 17897469.0.7 sequence was isolated and suncloned into into BamHI and XhoI treated pMelV5His to generate expression vector pMelV5His-17897469. The pMelV5His-17897469 vector was co-transfected with linearized baculovirus DNA (Bac-N-Blue) into Sf9 insect cells using liposome-mediated transfer as described by the manufacturer (Invitrogen). Briefly, transfection mixtures containing 4 ug of pMelV5His-17897469, 0.5 ug of Bac-N-Blue™ and InsectinPlus™ liposomes were added to 60 mm culture dishes seeded with 2 x 10^6 Sf9 cells, and incubated with rocking at 27°C for 4 hours. Fresh culture medium was added and cultures were further incubated for 4 days. The culture medium was then harvested and recombinant viruses were isolated using standard plaque purification procedures. Recombinant viruses expressing β–galactosidase as a marker were readily distinguished from non-recombinant viruses by visually inspecting agarose overlays for blue plaques. Viral stocks were generated by propagation on Sf9cells and screened for expression of 17897469 protein by SDS-PAGE and Western blot analyses (reducing conditions, anti-V5 antibody). See FIG 1.

EXAMPLE 5: CHROMOSOMAL LOCALIZATION OF TSP2

Radiation hybrid mapping using human chromosome markers was performed to define the chromosomal location of TSP2 nucleic acids of the invention. Mapping was performed generally as described in Steen, RG et al. (A High-Density Integrated Genetic Linkage and Radiation Hybrid Map of the Laboratory Rat, Genome Research 1999 (Published Online on [illegible]). A panel of 93 cell clones containing randomized

primers designed designed to specifically identify TSP2 nucleic acids of the invention. TSP2 was shown to localize to chromosome 19.

EXAMPLE 6: TISSUE EXPRESSION ANALYSIS OF TSRX NUCLEIC ACIDS

The quantitative expression of various clones was assessed in approximately 41 normal and 54 tumor samples by real time quantitative PCR (TAQMAN®) performed on a Perkin-Elmer Biosystems ABI PRISM® 7700 Sequence Detection System.

96 RNA samples were normalized to β-actin and GAPDH. RNA (~50 ng total or ~1 ng polyA+) was converted to cDNA using the TAQMAN® Reverse Transcription Reagents Kit (PE Biosystems, Foster City, CA; cat # N808-0234) and random hexamers according to the manufacturer's protocol. Reactions were performed in 20 ul and incubated for 30 min. at 48 C. cDNA (5 ul) was then transferred to a separate plate for the TAQMAN® reaction using β-actin and GAPDH TAQMAN® Assay Reagents (PE Biosystems; cat. #'s 4310881E and 4310884E, respectively) and TAQMAN® universal PCR Master Mix (PE Biosystems; cat # 4304447) according to the manufacturer's protocol. Reactions were performed in 25 ul using the following parameters: 2 min. at 50 C; 10 min. at 95 C; 15 sec. at 95 C 1 min. at 60 C (40 cycles). Results were recorded as CT values (cycle at which a given sample crosses a threshold level of fluorescence) using a log scale, with the difference in RNA concentration between a given sample and the sample with the lowest CT value being represented as 2 to the power of delta CT. The percent relative expression is then obtained by taking the reciprocal of this RNA difference and multiplying by 100. The average CT values obtained for β-actin and GAPDH were used to normalize RNA samples. The RNA sample generating the highest CT value required no further diluting, while all other samples were diluted relative to this sample according to their β-actin / GAPDH average CT values.

Normalized RNA (5 ul) was converted to cDNA and analyzed via TAQMAN® using One Step RT-PCR Master Mix Reagents (PE Biosystems; cat. # 4309169) and gene-specific primers according to the manufacturer's instructions. Probes and primers were designed for each assay according to Perkin Elmer Biosystem's *Primer Express* Software package (version 1) for Apple Computer's Macintosh Power PC using the nucleic acid sequences of the [illegible] primers constructed as shown in

range = 58°-60° C, primer optimal Tm = 59° C, maximum primer difference = 2° C, probe does not have 5' G, probe T$_m$ must be 10° C greater than primer T$_m$, amplicon size 75 bp to 100 bp. The probes and primers selected (see below) were synthesized by Synthegen (Houston, TX, USA). Probes were double purified by HPLC to remove uncoupled dye and evaluated by mass spectroscopy to verify coupling of reporter and quencher dyes to the 5' and 3' ends of the probe, respectively. Their final concentrations were, forward and reverse primers, 900 nM each, and probe, 200nM.

PCR was performed as follows, normalized RNA from each tissue and each cell line was spotted in each well of a 96 well PCR plate (Perkin Elmer Biosystems). PCR cocktails including two probes (TSRX-specific and another gene-specific probe multiplexed with the TSRX probe) were set up using 1X TaqMan® PCR Master Mix for the PE Biosystems 7700, with 5 mM MgCl2, dNTPs (dA, G, C, U at 1:1:1:2 ratios), 0.25 U/ml AmpliTaq Gold™ (PE Biosystems), and 0.4 U/l RNase inhibitor, and 0.25 U/l reverse transcriptase. Reverse transcription was performed at 48° C for 30 minutes followed by amplification PCR cycles as follows: 95° C 10 min, then 40 cycles of 95° C for 15 seconds, 60° C for 1 minute.

A summary of the expression results in cell lines is shown Tables 6 and 7. Expresion in the indicted cell line for the given TSRX sequence is presented as a percentage of expression relative to the reference transcript. Table 6 shows data using probe set AG67, whereas Table 7 shows data using probe set AG 813. High expression is found in adipose tissue, adrenal gland, fetal brain, normal brain cells, lymph node, fetal kidney, fetal liver, mammary gland, placenta, and testis. Expression is weak in most tumor cell lines except non-small cell lung cancer.

Tables 8 and 9 summarized the expression results in a surgical tissue sample panel set. Expression in the indicted tissue sample for the given TSRX sequence is presented as a percentage of expression relative to the reference transcript. Table 8 shows data using probe set AG67, whereas Table 9 shows data using probe set AG 813. The indicated in Tables 8 and 9 results higher expression is found in normal adjacent tissue as compared to the adjacent tumor. One exception is breast cancer metastases, in which a higher level of expression is observed as compared to the primary breast cancer.

Table 5

Probe Set	Primers Probes	SEQ ID NO:
Ag67	Forward 5'-GATGTCGTCTGGATTCCCAAA-3'	37
	Probe: Fam 5'-CCGTCCACATCTTCATCCAGGATCTGAAC-3' TAMRA	38
	Reverse 5'-GGGCCAAGTGACTGAGAGAGA-3'	39
Ag813	Forward 5'-TGTAGAATTTCCCACGGAAAG-3'	40
	Probe: FAM-5'-CACTGCACTTCTCTGAAGTCCTGGGA-3'-TAMRA	41
	Reverse 5'-TTGCAACACGGATGACTGT-3'	42

Table 6

Tissue	Relative Expression, %	Tissue	Relative Expression, %
Endothelial cells	0.2	Kidney (fetal)	65.1
Endothelial cells (treated)	6.3	Renal ca. 786-0	0.0
Pancreas	32.8	Renal ca. A498	7.5
Pancreatic ca. CAPAN 2	0.1	Renal ca. RXF 393	2.9
Adipose	62.4	Renal ca. ACHN	0.0
Adrenal gland	42.3	Renal ca. UO-31	2.2
Liver	11.4	Renal ca. TK-10	24.6
Salivary gland	0.1	Liver	24.3
Pituitary gland	27.4	Liver (fetal)	0.4
Brain (fetal)	1.0	Liver ca. (hepato blast) HepG2	0.0
Brain (adult)	5.0	Lung	14.7

Brain (hippocampus)	71.2	Lung ca. (small cell) NCI-H69	0.3
Brain (substantia nigra)	32.1	Lung ca. (s.cell var.) SHP-77	0.0
Brain (thalamus)	25.4	Lung ca. (large cell)NCI-H460	0.6
Brain (hypothalamus)	27.7	Lung ca. (non-sm. cell) A549	21.9
Spinal cord	26.1	Lung ca. (non-s.cell) NCI-H23	6.4
CNS ca. (glio/astro) U87-MG	0.0	Lung ca. (non-s.cell) HOP-62	3.7
CNS ca. (glio/astro) U-118-MG	0.2	Lung ca. (non-s.cl) NCI-H522	10.1
CNS ca. (astro) SW1783	0.3	Lung ca. (squam.) SW 900	9.3
CNS ca.* (neuro; met) SK-N-AS	0.2	Lung ca. (squam.) NCI-H596	0.0
CNS ca. (astro) SF-539	6.2	Mammary gland	41.2
CNS ca. (astro) SNB-75	7.5	Breast ca.* (pl. effusion) MCF-7	6.1
CNS ca. (glio) SNB-19	0.9	Breast ca.* (pl.ef) MDA-MB-231	6.0
CNS ca. (glio) U251	5.2	Breast ca.* (pl. effusion) T47D	7.1
CNS ca. (glio) SF-295	2.1	Breast ca. BT-549	9.9
Heart	11.8	Breast ca. MDA-N	9.2
Skeletal muscle	4.5	Ovary	14.9
[illegible]	4.5	Ovarian ca. OVCAR-3	0.0

Spleen	2[illegible].3	Ovarian ca. OVCAR-5	3.9
Lymph node	65.[illegible]	Ovarian ca. OVCAR-8	15.5
Colon (ascending)	7.0	Ovarian ca. IGROV-1	0.8
Stomach	13.9	Ovarian ca.* (ascites) SK-OV-3	0.3
Small intestine	16.8	Uterus	12.9
Colon ca. SW480	0.0	Placenta	21.5
Colon ca.* (SW480 met)SW620	0.0	Prostate	15.7
Colon ca. HT29	0.0	Prostate ca.* (bone met)PC-3	0.0
Colon ca. HCT-116	0.0	Testis	29.5
Colon ca. CaCo-2	0.4	Melanoma Hs688(A).T	4.5
Colon ca. HCT-15	0.4	Melanoma* (met) Hs688(B).T	7.0
Colon ca. HCC-2998	0.1	Melanoma UACC-62	0.1
Gastric ca.* (liver met) NCI-N87	0.0	Melanoma M14	0.1
Bladder	14.8	Melanoma LOX IMVI	0.8
Trachea	21.9	Melanoma* (met) SK-MEL-5	0.0
Kidney	1[illegible].2	Melanoma SK-MEL-28	0.0

ca. = carcinoma
* = established from metastasis
met = metastasis
[illegible]
[illegible]

squam = squamous
pl. eff = pleural effusion [illegible]
[illegible]
astro = astrocytoma
neuro = neuroblastoma

Table 7

Tissue	Relative Expression, %	Tissue	Relative Expression, %
Endothelial cells	0.0	Renal ca. A498	0.0
Endothelial cells (treated)	0.0	Renal ca. RXF 393	0.0
Pancreas	0.0	Renal ca. ACHN	0.0
Pancreatic ca. CAPAN 2	0.0	Renal ca. UO-31	0.0
Adipose	0.1	Renal ca. TK-10	5.8
Adrenal Gland (new lot*)	15.3	Liver	5.0
Thyroid	0.2	Liver (fetal)	1.5
Salavary gland	1.8	Liver ca. (hepatoblast) HepG2	0.0
Pituitary gland	9.0	Lung	2.3
Brain (fetal)	100.0	Lung (fetal)	9.9
Brain (whole)	15.2	Lung ca. (small cell) LX-1	0.0
Brain (amygdala)	11.4	Lung ca. (small cell) NCI-H69	0.0
Brain (cerebellum)	0.5	Lung ca. (s.cell var.) SHP-77	0.0
Brain (hippocampus)	23.2	Lung ca. (large cell) NCI-H460	0.0
Brain (thalamus)	3.2	Lung ca. (non-sm. Cell) A549	29.9
Spinal cord	0.2	Lung ca. (non-s.cell) NCI-H23	1.9
CNS ca. (glio/astro) U87-MG	0.0	Lung ca. (non-s.cell) HOP-62	4.2
CNS ca. (glio/astro) U-118-MG	0.0	Lung ca. (non-s.cell) NCI-H522	3.4
CNS ca. (astro) SF-539	0.0	Lung ca. (squam.) SW 900	0.2
CNS ca. (neuro; met) SK-N-AS	0.0	Lung ca. (squam.) NCI-H596	0.0
CNS ca. (astro) SF-539	0.3	Mammary gland	3.2

CNS ca. (glio) SNB-19	[illegible]	Breast ca.* (pl. ef) MDA-MB-231	[illegible]
CNS ca. (glio) U251	[illegible]	Breast ca.* (pl. effusion) T47D	0.2
CNS ca. (glio) SF-295	[illegible]	Breast ca. BT-549	[illegible]
Heart	7.6	Breast ca. MDA-N	0.0
Skeletal Muscle (new lot*)	0.7	Ovary	2.7
Bone marrow	0.5	Ovarian ca. OVCAR-3	0.0
Thymus	0.8	Ovarian ca. OVCAR-4	0.0
Spleen	1.2	Ovarian ca. OVCAR-5	0.2
Lymph node	0.2	Ovarian ca. OVCAR-8	2.6
Colorectal	0.0	Ovarian ca. IGROV-1	0.0
Stomach	1.3	Ovarian ca.* (ascites) SK-OV-3	0.0
Small intestine	7.4	Uterus	8.2
Colon ca. SW480	0.0	Placenta	3.5
Colon ca.* (SW480 met) SW620	0.0	Prostate	0.1
Colon ca. HT29	0.0	Prostate ca.* (bone met) PC-3	0.0
Colon ca. HCT-116	0.0	Testis	2.7
Colon ca. CaCo-2	0.0	Melanoma Hs688(A).T	0.9
83219 CC Well to Mod Diff (ODO3866)	0.1	Melanoma* (met) Hs688(B).T	0.8
Colon ca. HCC-2998	[illegible]	Melanoma M14	[illegible]
Gastric ca.* (liver met) NCI-N87	[illegible]	Melanoma LOX IMVI	[illegible]
Bladder	15.8	Melanoma* (met) SK-MEL-5	[illegible]
Trachea	[illegible]		
Kidney	[illegible]		

Renal ca. TS660	0.0

ca. = carcinoma
* = established from metastasis
met = metastasis
s cell var = small cell variant
non-sm = non-small
squam = squamous
pl. eff = pleural effusion
glio = glioma
astro = astrocytoma
neuro = neuroblastoma

Table 8

Tissue	Relative Expression, %	Tissue	Relative Expression, %
83786 Kidney Ca, Nuclear grade 2 (OD04338)	55.1	87475 Kidney NAT (OD04622-03)	8.4
83219 CC Well to Mod Diff (ODO3866)	11.0	87492 Ovary Cancer (OD04768-07)	0.0
83220 CC NAT (ODO3866)	3.5	87493 Ovary NAT (OD04768-08)	33.0
83221 CC Gr 2 rectosigmoid (ODO3868)	30.8	Bladder Cancer INVITROGEN A302173	1.7
83222 CC NAT (ODO3868)	11.0	Bladder Cancer Research Genetics RNA 1023	12.2
83235 CC Mod Diff (ODO3902)	39.6	Breast Cancer Clontech 91002a6	6.5
83236 CC NAT (ODO3902)	4.0	Breast Cancer INVITROGEN A302173	5.1
83237 CC Gr 2 ascend colon (ODO3921)	4.9	Breast Cancer Res Gen 1024	11.0
83238 CC NAT (ODO3921)	5.5	Breast NAT Clontech 91002a5	2.5
83254 Lung Met to Muscle		Breast NAT INVITROGEN	

83241 CC Hom Partial Hepatectomy (OD04309)	8.9	Gastric Cancer Clontech 9060395	23.3
83242 Liver NAT (OD04309)	98.6	Gastric Cancer Clontech 9060397	1.1
83255 Ocular Mel Metsto Liver (OD04310)	4.8	Gastric Cancer GENPAK 064005	32.8
83256 Liver NAT (OD04310)	41.5	Kidney Cancer Clontech 8120607	19.6
83787 Kidney NAT (OD04338)	78.5	Kidney Cancer Clontech 8120613	0.0
83788 Kidney Ca Nuclear grade 1 2 (OD04339)	21.3	Kidney Cancer Clontech 9010320	5.3
83789 Kidney NAT (OD04339)	28.5	Kidney NAT Clontech 8120608	2.8
83790 Kidney Ca Clear cell type (OD04340)	49.0	Kidney NAT Clontech 8120614	8.2
83791 Kidney NAT (OD04340)	24.5	Kidney NAT Clontech 9010321	35.9
83792 Kidney Ca Nuclear grade 3 (OD04341)	0.9	Liver Cancer GENPAK 064003	19.0
83793 Kidney NAT (OD04341)	15.2	Liver Cancer Research Genetics RNA 625	95.6
84136 Lung Malignant Cancer (OD03126)	0.0	Liver Cancer Research Genetics RNA 626	20.2
4137 Lung NAT (OD03126)	35.1	NAT Stomach Clontech 9060359	0.2
4138 Lung NAT (OD04321)	1.8	NAT Stomach Clontech 9060394	12.5
4139 Melanoma Met to Lung (OD04321)	0.9	NAT Stomach Clontech 9060396	7.4
4140 Prostate Cancer (OD04410)	49.2	Normal Bladder GENPAK 061001	54.1
4141 Prostate NAT (OD04410)	[illegible]	Normal Breast GENPAK 061002	21.2
[illegible]	[illegible]	[illegible] GENPAK 061005	25.2

		62005	
84875 Lung Cancer (OD04565	0.0	Normal Liver GENPAK 061009	72.7
84877 Breast Cancer (OD04566)	27.9	Normal Lung GENPAK 061019	4.5
85950 Lung Cancer (OD04237-01)	0.0	Normal Ovary Res. Gen.	19.1
85970 Lung NAT (OD04237-02)	2.4	Normal Prostate Clontech A+ 6546-1	0.4
85973 Kidney Cancer (OD04450-01)	0.0	Normal Stomach GENPAK 061017	0.0
85974 Kidney NAT (OD04450-03)	47.6	Normal Thyroid Clontech A+ 6570-1**	0.0
85975 Breast Cancer (OD04590-01)	4.3	Normal Uterus GENPAK 061018	11.0
85976 Breast Cancer Mets (OD04590-03)	16.6	Ovarian Cancer GENPAK 064008	20.0
87070 Breast Cancer Metastasis (OD04655-05)	61.6	Paired Liver Cancer Tissue Research Genetics RNA 6004-T	66.9
87071 Bladder Cancer (OD04718-01)	0.0	Paired Liver Cancer Tissue Research Genetics RNA 6005-T	12.9
87072 Bladder Normal Adjacent (OD04718-03)	2.7	Paired Liver Tissue Research Genetics RNA 6004-N	0.9
87073 Prostate Cancer (OD04720-01)	64.2	Paired Liver Tissue Research Genetics RNA 6005-N	32.3
87074 Prostate NAT (OD04720-02)	7.7	Thyroid Cancer GENPAK 064010	9
87472 Colon mets to lung (OD04451-01)	29.9	Thyroid Cancer INVITROGEN A302152	9.0
		Thyroid NAT INVITROGEN	

01	
	genomic DNA control.

"NAT" indicates surgically obtained normal adult tissue from a region immediately adjacent the tumor.

Table 9

Tissue	Relative Expression, %	Tissue	Relative Expression, %
87486 Kidney Ca, Nuclear grade 2 (OD04338)	47.0	87475 Kidney NAT (OD04622-03)	4.8
87219 CC Well to Mod Diff (ODO3866)	2.5	87492 Ovary Cancer (OD04768-07)	0.0
87220 CC NAT (ODO3866)	1.9	87493 Ovary NAT (OD04768-08)	5.7
87221 CC Well to Mod Diff rectosigmoid (ODO3868)	28.7	Bladder Cancer INVITROGEN A302173	1.3
87222 CC NAT (ODO3868)	4.2	Bladder Cancer Research Genentes RNA 1023	10.5
85235 CC Mod Diff (ODO3920)	5.8	Breast Cancer Clontech 9100266	5.4
85236 CC NAT (ODO3920)	2.3	Breast Cancer INVITROGEN A2034734	2.2
85237 CC Well to Mod Diff (ODO3921)	4.5	Breast Cancer Res. Gen. 1024	14.1
85238 CC NAT (ODO3921)	3.0	Breast NAT Clontech 9100265	8.1
85239 Lung Met to Muscle (OD04286)	1	Breast NAT INVITROGEN A2034734	0.3

83241 CC from Partial Hepatectomy (OD04308)	1.4	Gastric Cancer Clontech 9060395	8.7
83242 Liver NAT (OD04309)	12.9	Gastric Cancer Clontech 9060397	0.1
83255 Ocular Mel Met to Liver (ODO4310)	5.0	Gastric Cancer GENPAK 064005	12.3
83256 Liver NAT (OD04310)	27.7	Kidney Cancer Clontech 8120607	6.1
83787 Kidney NAT (OD04338)	10.8	Kidney Cancer Clontech 8120613	0.0
83788 Kidney Ca Nuclear grade 1/2 (OD04339)	13.6	Kidney Cancer Clontech 9010320	4.0
83789 Kidney NAT (OD04339)	22.2	Kidney NAT Clontech 8120608	6.2
83790 Kidney Ca, Clear cell type (OD04340)	33.0	Kidney NAT Clontech 8120614	4.0
83791 Kidney NAT (OD04340)	23.3	Kidney NAT Clontech 9010321	11.7
83792 Kidney Ca, Nuclear grade 3 (OD04348)	2.1	Liver Cancer GENPAK 064003	16.2
83793 Kidney NAT (OD04348)	30.4	Liver Cancer Research Genetics RNA 1025	88.9
84136 Lung Malignant Cancer (OD[illegible])	[illegible]	Liver Cancer Research Genetics RNA 1026	[illegible]
84137 Lung NAT (OD03126)	1.8	NAT Stomach Clontech 9060359	1.8
84138 Lung NAT (OD04321)	[illegible]	NAT Stomach Clontech 9060394	[illegible]

(OD04410)		061001	
84243 Prostate NAT (OD04410)	63.5	Normal Breast GENPAK 061019	7.1
84871 Lung Cancer (OD04404)	0.0	Normal Colon GENPAK 061003	5.9
84872 Lung NAT (OD04404)	0.0	Normal Kidney GENPAK 061005	10.8
84875 Lung Cancer (OD04565)	3.7	Normal Liver GENPAK 061009	36.1
84877 Breast Cancer (OD04566)	8.6	Normal Lung GENPAK 061010	1.4
85950 Lung Cancer (OD04237-01	0.0	Normal Ovary Res. Gen.	15.2
85970 Lung NAT (OD04237-02)	1.6	Normal Prostate Clontech A+ 6546-1	2.2
85973 Kidney Cancer (OD04450-01)	0.0	Normal Stomach GENPAK 061017	0.0
85974 Kidney NAT (OD04450-03)	25.9	Normal Thyroid Clontech A+ 6570-1**	0.0
85975 Breast Cancer (OD04590-01)	0.9	Normal Uterus GENPAK 061018	36.1
85976 Breast Cancer Met (OD04590-03)	2.1	Ovarian Cancer GENPAK 061012	17.2
[illegible]		Paired Lung Cancer Tissue Research Genetics RNA 6004-	
Metastasis (OD04255-01)	47.1	2	3.9
[illegible]		Paired Liver Cancer Tissue Research Genetics RNA 6005-	
[illegible]	1	2	2.1

8573 Prostate Cancer (OD04720-01)	7.9	Paired Liver Tissue Research Genetics RNA 6005-N	24.5
8574 Prostate NAT (OD04720-02)	1.4	Thyroid Cancer GENPAK 064010	9.1
8472 Colon mets to lung (OD04451-01)	1.7	Thyroid Cancer INVITROGEN A302152	2.0
8473 Lung NAT (OD04451-02)	11.3	Thyroid NAT INVITROGEN A302153	1.4
8474 Kidney Cancer (OD04e22-01)	4.1	Uterus Cancer GENPAK 064011	22.2
		genomic DNA control	9.6

"NAT" indicates surgically obtained normal adjacent tissue from a region immediately adjacent the tumor

OTHER EMBODIMENTS

While the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

What is claimed is:

1. An isolated polypeptide comprising an amino acid sequence selected from the group consisting of:

a) a mature form of the amino acid sequence given by SEQ ID NO: 2 and 4;

b) a variant of a mature form of the amino acid sequence given by SEQ ID NO: 2 and 4, wherein any amino acid in the mature form is changed to a different amino acid, provided that no more than 15% of the amino acid residues in the sequence of the mature form are so changed;

c) the amino acid sequence given by SEQ ID NO: 2 and 4;

d) a variant of the amino acid sequence given by SEQ ID NO: 2 and 4 wherein any amino acid specified in the chosen sequence is changed to a different amino acid, provided that no more than 15% of the amino acid residues in the sequence are so changed; and

e) a fragment of any of a) through d).

2. The polypeptide of claim 1 that is a naturally occurring allelic variant of the sequence given by SEQ ID NO: 2 and 4.

3. The polypeptide of claim 2, wherein the variant is the translation of a single nucleotide polymorphism.

4. The polypeptide of claim 1 that is a variant polypeptide described therein, wherein any amino acid specified in the chosen sequence is changed to provide a conservative substitution.

a) a mature form of the amino acid sequence given SEQ ID NO: 2 and 4;

b) a variant of a mature form of the amino acid sequence given by SEQ ID NO: 2 and 4 wherein any amino acid in the mature form of the chosen sequence is changed to a different amino acid, provided that no more than 15% of the amino acid residues in the sequence of the mature form are so changed;

c) the amino acid sequence given by SEQ ID NO: 2 and 4;

d) a variant of the amino acid sequence given by SEQ ID NO: 2 and 4, in which any amino acid specified in the chosen sequence is changed to a different amino acid, provided that no more than 15% of the amino acid residues in the sequence are so changed;

e) a nucleic acid fragment encoding at least a portion of a polypeptide comprising the amino acid sequence given by SEQ ID NO: 2 and 4 or any variant of said polypeptide wherein any amino acid of the chosen sequence is changed to a different amino acid, provided that no more than 10% of the amino acid residues in the sequence are so changed; and

f) the complement of any of said nucleic acid molecules.

6. The nucleic acid molecule of claim 5, wherein the nucleic acid molecule comprises the nucleotide sequence of a naturally occurring allelic nucleic acid variant.

7. The nucleic acid molecule of claim 5 that encodes a variant polypeptide, wherein the variant polypeptide has the polypeptide sequence of a naturally occurring polypeptide variant.

8. The nucleic acid molecule of claim 5, wherein the nucleic acid molecule comprises a single nucleotide polymorphism encoding said variant polypeptide.

9. The nucleic acid molecule of claim 5, wherein said nucleic acid molecule comprises a nucleotide sequence selected from the group consisting of:

a) the nucleotide sequence given by SEQ ID NO: 1 and 3;

b) a nucleotide sequence wherein one or more nucleotides in the nucleotide sequence given by SEQ ID NO: 1 and 3is changed from that given by the chosen sequence to a different nucleotide provided that no more than 15% of the nucleotides are so changed;

c) a nucleic acid fragment of the sequence given by SEQ ID NO: 1 and 3; and

d) a nucleic acid fragment wherein one or more nucleotides in the nucleotide sequence given by SEQ ID NO: 1 and 3 is changed from that given by the chosen sequence to a different nucleotide provided that no more than 15% of the nucleotides are so changed.

10. The nucleic acid molecule of claim 5, wherein said nucleic acid molecule hybridizes under stringent conditions to the nucleotide sequence given by SEQ ID NO: 1 and 3, or a complement of said nucleotide sequence.

11. The nucleic acid molecule of claim 5, wherein the nucleic acid molecule comprises a nucleotide sequence in which any nucleotide specified in the coding sequence of the chosen nucleotide sequence is changed from that given by the chosen sequence to a different nucleotide provided that no more than 15% of the nucleotides in the chosen coding sequence are so changed, an isolated second polynucleotide that is a complement of the first polynucleotide, or a fragment of any of them.

12. A vector comprising the nucleic acid molecule of claim 11.

13. [illegible] of claim 12 [illegible] operably linked to said nucleic

14. A cell comprising the vector of claim 12.

15. An antibody that binds immunospecifically to the polypeptide of claim 1.

16. The antibody of claim 15, wherein said antibody is a monoclonal antibody.

17. The antibody of claim 15, wherein the antibody is a humanized antibody.

18. A method for determining the presence or amount of the polypeptide of claim 1 in a sample, the method comprising:

(a) providing said sample;

(b) introducing said sample to an antibody that binds immunospecifically to the polypeptide; and

(c) determining the presence or amount of antibody bound to said polypeptide,

thereby determining the presence or amount of polypeptide in said sample.

19. A method for determining the presence or amount of the nucleic acid molecule of claim 5 in a sample, the method comprising:

(a) providing said sample;

(b) introducing said sample to a probe that binds to said nucleic acid molecule; and

(c) determining the presence or amount of said probe bound to said nucleic acid molecule.

20. A method of identifying an agent that binds to the polypeptide of claim 1, the method comprising:

(a) introducing said polypeptide to said agent; and

(b) determining whether said agent binds to said polypeptide.

21. A method for identifying a potential therapeutic agent for use in treatment of a pathology, wherein the pathology is related to aberrant expression or aberrant physiological interactions of the polypeptide of claim 1, the method comprising:

(a) providing a cell expressing the polypeptide of claim 1 and having a property or function ascribable to the polypeptide;

(b) contacting the cell with a composition comprising a candidate substance; and

(c) determining whether the substance alters the property or function ascribable to the polypeptide;

whereby, if an alteration observed in the presence of the substance is not observed when the cell is contacted with a composition devoid of the substance, the substance is identified as a potential therapeutic agent.

22. A method for modulating the activity of the polypeptide of claim 1, the method comprising introducing a cell sample expressing the polypeptide of said claim with a compound that binds to said polypeptide in an amount sufficient to modulate the activity of the polypeptide.

which such treatment or prevention is desired in an amount sufficient to treat or prevent said pathology in said subject.

24. The method of claim 23, wherein said subject is a human.

25. A method of treating or preventing a pathology associated with the polypeptide of claim 1, said method comprising administering to a subject in which such treatment or prevention is desired a TSRX nucleic acid in an amount sufficient to treat or prevent said pathology in said subject.

26. The method of claim 25, wherein said subject is a human.

27. A method of treating or preventing a pathology associated with the polypeptide of claim 1, said method comprising administering to a subject in which such treatment or prevention is desired a TSRX antibody in an amount sufficient to treat or prevent said pathology in said subject.

28. The method of claim 15, wherein the subject is a human.

29. A pharmaceutical composition comprising the polypeptide of claim 1 and a pharmaceutically acceptable carrier.

30. A pharmaceutical composition comprising the nucleic acid molecule of claim 5 and a pharmaceutically acceptable carrier.

31. A pharmaceutical composition comprising the antibody of claim 15 and a pharmaceutically acceptable carrier.

32. A kit comprising in one or more containers, the pharmaceutical composition of claim 29.

33. A kit comprising in one or more containers, the pharmaceutical composition of claim 30.

34. A kit comprising in one or more containers, the pharmaceutical composition of claim 31.

35. The use of a therapeutic in the manufacture of a medicament for treating a syndrome associated with a human disease, the disease selected from a pathology associated with the polypeptide of claim 1, wherein said therapeutic is the polypeptide of claim 1.

36. The use of a therapeutic in the manufacture of a medicament for treating a syndrome associated with a human disease, the disease selected from a pathology associated with the polypeptide of claim 1, wherein said therapeutic is a TSRX nucleic acid.

37. The use of a therapeutic in the manufacture of a medicament for treating a syndrome associated with a human disease, the disease selected from a pathology associated with the polypeptide of claim 1, wherein said therapeutic is a TSRX antibody.

a) administering a test compound to a test animal at increased risk for a pathology associated with the polypeptide of claim 1, wherein said test animal recombinantly expresses the polypeptide of claim 1;

b) measuring the activity of said polypeptide in said test animal after administering the compound of step (a); and

c) comparing the activity of said protein in said test animal with the activity of said polypeptide in a control animal not administered said polypeptide, wherein a change in the activity of said polypeptide in said test animal relative to said control animal indicates the test compound is a modulator of latency of, or predisposition to, a pathology associated with the polypeptide of claim 1.

39. The method of claim 38, wherein said test animal is a recombinant test animal that expresses a test protein transgene or expresses said transgene under the control of a promoter at an increased level relative to a wild-type test animal, and wherein said promoter is not the native gene promoter of said transgene.

40. A method for determining the presence of or predisposition to a disease associated with altered levels of the polypeptide of claim 1 in a first mammalian subject, the method comprising:

a) measuring the level of expression of the polypeptide in a sample from the first mammalian subject; and

b) comparing the amount of said polypeptide in the sample of step (a) to the amount of the polypeptide present in a control sample from a second mammalian subject known not to have, or not to be predisposed to, said disease;

wherein an alteration in the expression level of the polypeptide in the first subject as compared

41. A method for determining the presence of or predisposition to a disease associated with altered levels of the nucleic acid molecule of claim 5 in a first mammalian subject, the method comprising:

a) measuring the amount of the nucleic acid in a sample from the first mammalian subject; and

b) comparing the amount of said nucleic acid in the sample of step (a) to the amount of the nucleic acid present in a control sample from a second mammalian subject known not to have or not be predisposed to, the disease,

wherein an alteration in the level of the nucleic acid in the first subject as compared to the control sample indicates the presence of or predisposition to the disease.

42. A method of treating a pathological state in a mammal, the method comprising administering to the mammal a polypeptide in an amount that is sufficient to alleviate the pathological state, wherein the polypeptide is a polypeptide having an amino acid sequence at least 95% identical to a polypeptide comprising the amino acid sequence given by SEQ ID NO: and 4 or a biologically active fragment thereof.

43. A method of treating a pathological state in a mammal, the method comprising administering to the mammal the antibody of claim 15 in an amount sufficient to alleviate the pathological state.

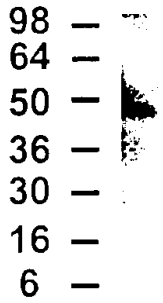


FIG 1.

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

A fragment of the ISRN polypeptide, polynucleotide or antibody. The invention additionally provides methods in which the ISRN polypeptide, polynucleotide and antibody are utilized in the detection and treatment of a broad range of pathological states, as well as to other uses.

INTERNATIONAL SEARCH REPORT

Inter onal Application No
PCT/US 00/26432

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/12 C07K14/47 C07K16/18 G01N33/53 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

BIOSIS, EMBL, STRAND, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE EMBL 'Online! Sequence AI131032, 23 September 1998 (1998-09-23) R STRAUSBERG: "H. sapiens cDNA clone IMAGE 1706639 3', Thrombospondin like, contains TAR1.t1 TAR1 repetitive element" XP002163036 encoded sequence is 100% identical to aa 465-629 in SEQ ID NO:2 and aa 296-461 in SEQ ID NO:4 --- -/--	5-11

[X] Further documents are listed in the continuation of box C.

[ ] Patent family members are listed in annex

* Special categories of cited documents :

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 00/26432

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE EMBL 'Online! Sequence AI673039; 19 May 1999 (1999-05-19) R STRAUSBERG "H. sapiens cDNA clone IMAGE:2345448 3', disintegrin and metalloprotease with thrombospondin motifs; contains TAR1.t1.TAR1 repetitive element" XP002163037 encoded aa sequence is 100% identical to aa 479-635 in SEQ ID NO:2 and aa 310-466 in SEQ ID NO:4 ---	5-11
X	DATABASE EMBL 'Online! Sequence AW027573; 17 September 1999 (1999-09-17) R STRAUSBERG: "H.sapiens cDNA clone IMAGE:2535323 3'" XP002163038 Encoded sequence is 99.5% identical to aa 415-626 in SEQ ID NO:2 and aa 246-467 in SEQ ID NO:4 ---	5-11
A	HURSKAINEN TIINA L ET AL: "ADAM-TS5, ADAM-TS6, and ADAM-TS7, novel members of a new family of zinc metalloproteases: General features and genomic distribution of the Adam-TS family." JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 274, no. 36, pages 25555-25563, XP002158991 ISSN: 0021-9258 figure 2B -----	1-13